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The Role of Time Variant Resistance and Electromotive Force in Ionic Systems Related to Cell Membranes: The Excitabilty Properties of Frog Skin and Toad Bladder

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THE ROLE OF TIME VARIANT RESISTANCE AND ELECTROMOTIVE FORCE IN IONIC SYSTEMS RELATED TO CELL MEMBRANES: THE EXCITABILITY PROPERTIES OF FROG SKIN AND TOAD BLADDER

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PREFACE

The purpose of this paper is to examine in a somewhat principled and critical manner the physical implications of the ionic theory of resting and action potentials across cell membranes. It is our aim to consider and discuss how potential differences can arise in certain well defined ionic systems and then to apply this analysis to the biological membrane. From these considerations, we shall see that there are two broad physical classes to which the cell membrane could belong, and we shall argue from both the theoretical and experimental studies presented in this paper that a logical, consistent, and (hopefully) convincing case can be made for one of them. It is hoped that this work will serve to narrow and direct attention to the relevant class of systems and phenomena which should be considered by those interested in the physicochemical origins of electrical excitability.

It is a great pleasure for me to express my thanks to Dr. Detlev W. Bronk for having enabled me to spend the last four years at the Rockefeller Institute. During these years I have had the unique opportunity not only of being associated with a faculty containing a rather high density of outstanding investigators, but also, and more to the point, of finding the members of this faculty accessible and always willing to discuss the various problems which have concerned me. In this regard, I must particularly thank Drs. Frank Brink, Clarence Connelly, Frederick Dodge, and Paul Hurlbut of the Biophysics Department for having provided a most stimulating environment in which to work; Drs. Mark Kac and George Uhlenbeck for their patience and efforts in clarifying for me some of the basic concepts of statistical and quantum physics; and Dr. Lewis Longsworth for enlightening discussions, too

numerous to count, on electrochemical problems. Above all, I wish to thank my friend and research advisor, Dr. Alexander Mauro, not only for his counsel, advice, and enlightenment about the many problems in electrophysiology and related subjects, but also for having taught me by the example of his own work the meaning of careful and critical (albeit at times emotional) scientific inquiry.

Finally, with regard to the physical preparation of the present opus, I wish to acknowledge my considerable indebtedness to Mr. Peter Rich for having employed his artistic and photographic talents in constructing all of the figures in the text, Miss Sonia Wohl for having checked and corrected the bibliography, Miss Marie Hall for translating indecipherable scribblings into legible typewritten drafts, and Miss Didi Bottemanne for typing, so elegantly, the final manuscript.

SUMMARY

Central to the modern theory of the electrical properties of cells is the permeability of the plasma membrane to the ions passing across this structure; it has been our purpose to explore, both theoretically and experimentally, the physical implications of this point-of-view. The cornerstone of our analysis has been the fundamental flux equations of Nernst and Planck, which describe the movement of ions in solution subject to gradients of electrochemical potential. As a direct consequence of these equations, we have seen that the potential difference between two points in a solution is the sum of two terms: an intrinsic electromotive force (emf.) associated with the gradients of ionic concentrations, and an ohmic potential difference (IR drop) arising when current flows through the system. In order to illustrate the meaning and significance of these two terms, we have considered the voltage-current relationships in certain synthetic membrane systems; namely, the homogeneous uncharged membrane, the homogeneous fixed-charge membrane, and the two-layer "sandwich" membrane consisting of a positive and negative fixed-charge lattice in series. Our analysis of these systems enabled us to bring up for discussion the important concepts of non-linear voltage characteristics, slope and chord resistance, time-variant resistance and emf., and rectification. We have noted that in the sandwich membrane of high fixed-charge density, the membrane "resistance" is primarily the result of emf.'s generated in the face of current flow, and we have demonstrated the behavior of such membranes experimentally.

We then turned our attention to a more direct consideration of the ionic theory of electrical events in cells, and in particular we focussed upon the "equivalent circuit" used to describe the electrical characteristics of the plasma membrane. In order to determine what such an equivalent circuit representation can mean physically, we returned to the basic flux

equations and have considered how these equations can be interpreted in electrical language. From these considerations, we have seen that two, quite different equivalent circuits can be written for a homogeneous membrane separating ionic solutions. Of particular significance is the fact that one of these circuits, which has the same form as that used for the plasma membrane, can also pertain to a mosaic membrane consisting of spatially separate regions of ionic selectivity. We have discussed the point that for a homogeneous membrane the essence of the action potential is a time-variant emf. while for a mosaic membrane it is a time-variant resistance.

This ambiguity in the meaning of the equivalent circuit led us to the experimental study of the electrical excitability of isolated frog skin and toad bladder. We have seen that when current of proper polarity and sufficient intensity is passed across these structure, an "all-or-none" action potential with a sharp threshold and a prolonged refractory period is elicited. We emphasized that interruption of the current during any point in the action potential abolishes the response, and we have shown, through appropriate bridge measurements, that this is a consequence of the fact that the action potential results from a modulation of the current flow by a time-variant resistance.

We then investigated some of the parameters affecting this active response. The result of varying the ionic composition of the medium was studied in the frog skin, and it was found that the response is relatively insensitive to changes in the solution bathing the inner surface, but rapidly and reversibly affected by changes in the outer solution, particularly with regard to the replacement of sodium by potassium and with respect to variations in the calcium concentration. It was also observed that the resistance of the skin and the action potential across it are reversibly altered by metabolic inhibitors and that these alterations occur independently of any changes in the intrinsic emf. across the system.

From the finding that the action potential in frog skin and toad bladder is the result of a time-variant resistance, we have argued that this same phenomenon can be the basis of electrical excitability in general. This necessitates attributing real physical significance to the equivalent circuit representing the plasma membrane; that is, the plasma membrane must be a mosaic structure of spatially separate permselective regions.

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INTRODUCTION

Separating the interior of a cell from the surrounding milieu is a thin stratum of matter of approximately 50 to 100 Å in thickness known as the plasma membrane. It is generally recognized that the electrical properties of all cells, i.e. the resting potential, impedance, current-voltage characteristic, etc. are attributable to this structure; in so-called excitable cells such as muscle and nerve, the characteristic event known as the "action potential" is an additional electrical manifestation of the plasma membrane. The elucidation of the physicochemical events occurring within the cell membrane, which underlie the electrical responses of the cell, is the central problem of electrophysiology. In attempting to gain understanding of this complex electrochemical machine, we have been led to the examination, both theoretical and experimental, of the electrical properties of certain synthetic membranes, along with an investigation of two specific "excitable" biological systems. It is with the results of these studies that this paper will deal.

It will perhaps be useful, before proceeding, to outline briefly the program which we shall pursue in the following exposition, and to indicate the point-of-view that will be adopted. The first part of this paper will be devoted to an exposition of the current-voltage relations in some artificial membrane systems; in particular, our study shall focus on membranes containing fixed-charged groups. While we shall attempt to indicate the possible relevance of the properties of these membranes to the plasma membrane, it should be clearly understood that there is no intended implication that we have proved that the physical mechanisms operating in the synthetic systems are the same as those accounting for the characteristics of the biological membrane. Rather, the philosophy of this approach is that our understanding of the behavior of some "simple", well defined model systems will be useful in focussing attention on certain physical facts of life which may be of importance in the plasma membrane. In fact, the most interesting aspect of these membranes is that their somewhat subtle and complex electrical characteristics arise purely from the presence of fixed charges in various spatial arrangements, and hence the analysis of these systems serves to demonstrate the wealth of

phenomena which can arise merely from the existence of charge groups in a membrane, without the necessity of introducing such concepts as steric hindrance, specific carriers, or various other phenomena which may be operating in the cell membrane.

The second part of this paper will open with a discussion of the present-day party line on the axonal membrane; in particular, we shall be concerned with the "equivalent circuit" used to represent the electrical properties of this membrane. We shall see that this circuit is applicable to two broad classes of membranes; namely, homogeneous membranes and mosaic membranes. Since, as we shall show, the physical basis for the electrical characteristics of these two classes of membranes is quite different, it becomes important to decide to which class the axonal membrane belongs. This will lead us to a treatment of the electrical excitability of frog skin and toad bladder, which, we shall argue, will give us an insight into the nature of the axonal membrane. In addition, the electrical responses of these structures will be seen to have an intrinsic interest in themselves.

PART I

A. The Flux Equations

Preparatory to our treatment of the electrical properties of membrane systems, we shall discuss the flux equations governing the transport of ions in solution. For the sake of simplicity, we shall confine our attention to an isothermal solution of univalent ions in which the only "forces" operating on the ionic species are gradients of their chemical and electrical potential. Thus, in particular, mass flow of solvent is excluded from consideration. We assume that we can take, without serious error, the activity coefficients of all species to be 1, so that our equations will involve the concentrations of the ions rather than their activities; furthermore, we regard the ionic mobilities as constant; that is, not a function of concentration. Finally, for algebraic convenience, we shall consider the concentrations and potential to vary in only one direction (x) in space. Then, the fluxes per unit area, ϕ_j^+ and ϕ_k^-, of the j'th cation and k'th anion, respectively, at any point in the solution are given by the fundamental flux equations of Nernst and Planck (Nernst, 1888, 1889; Planck, 1890 a, b):

$$\phi_j^+ = - u_j c_j^+ \left(RT \frac{1}{c_j^+} \frac{\partial c_j^+}{\partial x} + F \frac{\partial \psi}{\partial x} \right) \tag{1a}$$

$$\phi_k^- = - v_k c_k^- \left(RT \frac{1}{c_k^-} \frac{\partial c_k^-}{\partial x} - F \frac{\partial \psi}{\partial x} \right) \tag{1b}$$

where,

u_j = the mobility of the j'th cation

v_k = the mobility of the k'th anion

c_j^+, c_k^- = the concentration of the j'th and k'th ions, respectively, at any point x in the solution at time t

ψ = the electrical potential at any point x in the solution at time t

R = the gas constant

T = the absolute temperature

F = the Faraday constant

These equations will form the starting point for all subsequent discussions, and we shall make no attempt to derive or justify them from more basic considerations.* We note in passing that for uncharged species the $\frac{\partial \psi}{\partial x}$ term will be absent, in which case equation (1) reduces to Fick's Law.

In order to appreciate how $\frac{\partial \psi}{\partial x}$ arises in ionic solutions, let us sum (1a) and (1b) over all species and subtract to obtain:

$$\sum_j \phi_j^+ - \sum_k \phi_k^- = RT\left(\frac{\partial V}{\partial x} - \frac{\partial U}{\partial x}\right) - F(U+V)\frac{\partial \psi}{\partial x} \qquad (2)$$

where we have followed the Planck notation,

$$U = \sum_j u_j c_j^+ \qquad V = \sum_k v_k c_k^-$$

Recognizing that the electrical current, I, at any point in the solution is, by definition,

$$I \equiv \sum_j \phi_j^+ - \sum_k \phi_k^-$$

equation (2) upon rearrangement becomes:

$$-\frac{\partial \psi}{\partial x} = I\frac{1}{F(U+V)} + \frac{RT}{F}\frac{\frac{\partial (U-V)}{\partial x}}{(U+V)} \qquad (3a)$$

or rewriting this in slightly different form,

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*These equations in one sense are almost intuitively obvious, since they simply state that each ion moves as a consequence of the gradient of its chemical potential

$$RT\left(\frac{1}{c}\frac{\partial c}{\partial x}\right)$$

and of the gradient of the electrical potential

$$\left(F\frac{\partial \psi}{\partial x}\right).$$

What is not so obvious is why the same mobility, u, multiplies both terms, for the former term is a statistical "force", while the latter is a true force acting on each individual ion. Nevertheless, the electrical mobility and diffusional mobility are intimately related, as can be proved with varying degrees of rigor from Brownian motion theory (Einstein, 1905; Uhlenbeck and Ornstein, 1930).

$$-d\psi = I\frac{dx}{F(U+V)} + \frac{RT}{F}\frac{d(U-V)}{(U+V)} \qquad (3b)$$

where it is understood that we are considering the system at a given instant in time and that in general ψ, I, U, and V are functions not only of x but also of t. Now, since the specific resistivity of a slab of solution lying between x and x+dx is

$$\frac{dx}{F(U+V)},$$

the physical interpretation of equation (3b) is clear; namely, that the potential difference, $-d\psi$, between x and x+dx is the sum of the IR drop,

$$I\frac{dx}{F(U+V)},$$

between these two points and an emf. term,

$$\frac{RT}{F}\frac{d(U-V)}{(U+V)},$$

arising from the gradient of the concentrations of the ionic species.* Note, that when I = 0, this second term will be the only one contributing to the potential difference, while if the solution is homogeneous, that is, there are no gradients of ionic concentrations, then d(U - V) = 0, and the only potential differences arising in the solution will be due to IR drops. If we assume that I does not vary with x, (that is, the divergence of the current is zero), then (3b) may be formally integrated to give:

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*The expression

$$\frac{RT}{F}\frac{d(U-V)}{(U+V)}$$

can also be derived from quasi-thermodynamic reasoning (MacInnes; Denbigh) and is usually written in the form:

$$\frac{RT}{F}\sum_{j,k}\left(t_j^+ d\mu_j - t_k^- d\mu_k\right),$$

where t_j is the transference number of the j'th ion and μ_j its chemical potential.

$$-\int_{\psi_1}^{\psi_2} d\psi = -(\psi_2 - \psi_1) = I \int_1^2 \frac{dx}{F(U+V)} + \frac{RT}{F} \int_1^2 \frac{d(U-V)}{(U+V)} \qquad (3c)$$

and from the discussion above we see that the potential difference between two points in the solution is the sum of the IR drop between these points and a diffusion emf. given by

$$\frac{RT}{F} \int_1^2 \frac{d(U-V)}{(U+V)} \quad .$$

Equation (3), which was originally derived by Planck (1890 a), is an extremely general result, being a simple algebraic consequence of the flux equations, and consequently it has the same range of validity. In particular, it should be noted that this relationship applies equally well to space charge regions as well as to electroneutral regimes. The reader should not, however, be misled by the elegant form of (3) into thinking that it is a simple matter to calculate the potential function in an ionic solution. One must remember that in order to integrate (3), the concentrations of all ions must be known as functions of x. As we shall see shortly when we turn to the task of determining the potential difference across a membrane, this can be a formidable problem.

B. The Homogeneous-Uncharged Membrane

The homogeneous-uncharged membrane is characterized by the absence of any interaction between the ions and the membrane; its only function is to present a convection-free barrier, freely permeable to ions and water, across which boundary conditions can be maintained by effective stirring. Thus, the mobilities of all ions are the same within the membrane as in free solution; furthermore, mass flow of water is considered to be negligible. In order that the boundary conditions be kept constant, it is assumed that the membrane separates well-stirred solutions of "infinite" volumes (Figure 1). (In this

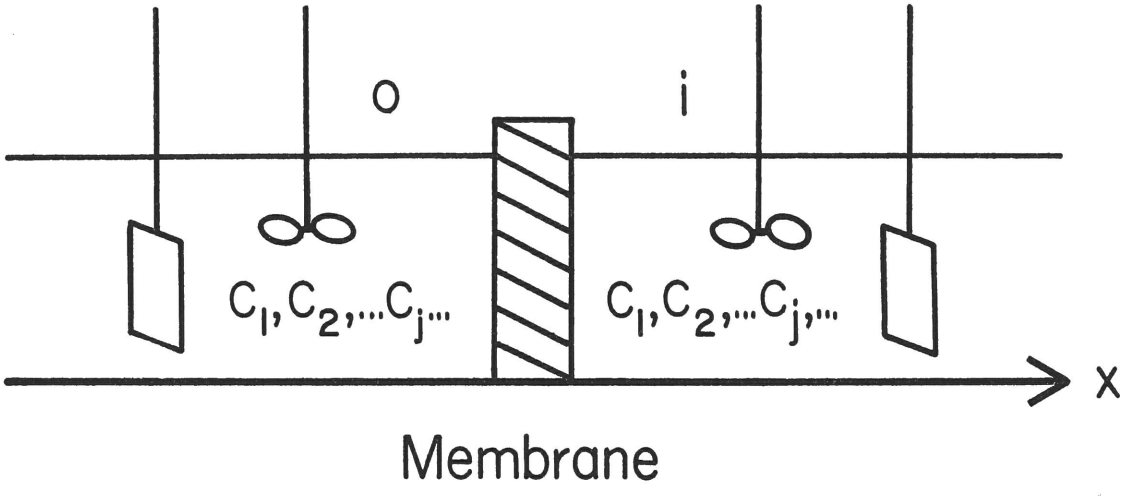

Figure 1. Homogeneous membrane separating two solutions of univalent ions. The solutions are essentially "infinite" and well stirred so that the boundary conditions are maintained. A pair of electrodes are present by means of which current can be passed across the membrane.

figure there is included a pair of electrodes by means of which current may be passed across the membrane.) The general steady state analysis of this system which we have described above has been carried out completely by Planck (1890 b); we shall deal only with two special cases, which will suffice to introduce certain ideas and concepts that will be needed later. It is to be understood that our discussion, unless otherwise indicated, will be confined to the steady state situation, which is characterized by a constant flux, ϕ_j, of the j'th ion at each point in the membrane.

1. The single salt case. For this case the membrane separates solutions of a single salt (for example, NaCl) at concentrations c_i and c_0.* The problem here, as in the most general case, is to solve the flux equations [equations (1)] for the concentrations of each ion as a function of x; that is, we wish to determine the "concentration profiles" of the ions within the membrane. Once this has been done, all the relevant electrical properties can be calculated by straightforward integration. However, equations (1) plus the boundary conditions are not sufficient to determine a unique solution of this problem; one more condition is necessary. This is the so-called "condition of electroneutrality," which states that at any point x within the membrane, the total concentrations of cations and anions are equal, i.e.:

*Throughout this paper we shall arbitrarily call the solution on one side of the membrane the inner solution and the solution on the other side the outer solution. The concentrations of ions in the inner and outer solutions will be designated with the subscripts i and 0, respectively. (Alternatively, we shall sometimes use the subscripts 1 and 2.) Thus, $(c_j)_i$ is the concentration of the j'th ion in the inner solution and $(c_j)_0$ is its concentration in the outer solution.

$$\sum_j c_j^+ = \sum_k c_k^- \quad . \tag{4}^*$$

For the single salt case, the flux equations are:

$$\phi^+ = - uc^+ \left(RT \frac{1}{c^+} \frac{dc^+}{dx} + F \frac{d\psi}{dx} \right) \tag{6a}$$

$$\phi^- = - vc^- \left(RT \frac{1}{c^-} \frac{dc^-}{dx} - F \frac{d\psi}{dx} \right) \tag{6b}$$

while the electroneutrality condition is:

$$c^+ = c^- = c \tag{7}$$

Substituting (7) into (6) we have the two equations:

$$A \equiv - \frac{\phi^+}{u} = RT \frac{dc}{dx} + Fc \frac{d\psi}{dx}$$

$$B \equiv - \frac{\phi^-}{v} = RT \frac{dc}{dx} - Fc \frac{d\psi}{dx}$$

which upon addition give:

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*Equation (4) deserves some comment. At first glance, it would seem to imply that in the absence of current flow there would be no gradient of potential within the membrane, since the only way an electric field could be created is through the existence of space charge regions, and it is just this mechanism that is precluded by (4). Thus, the "correct" relation that should be added to the flux equations must not be the condition of electric neutrality, but rather Poisson's equation:

$$\frac{d^2\psi}{dx^2} = - \frac{4\pi}{\epsilon} F \left(\sum_j c_j^+ - \sum_k c_k^- \right) \tag{5}$$

where ϵ is the dielectric constant of the medium. While all of this is true enough, it turns out that the deviation from electric neutrality is so small that no serious error is introduced in using (4) rather than Poisson's equation. (For a discussion of this point, see Planck.) This is indeed fortunate, for while equations (1) plus (4) can be solved in closed form, equations (1) plus (5) form a set that is analytically intractible. (It may be remarked here that when we begin to consider membranes containing fixed-charges, we shall not be able to so glibly apply the electroneutrality condition, and shall, in fact, have to deal explicitly with Poisson's equation.)

$$(A + B) = 2RT \frac{dc}{dx} \quad . \tag{8}$$

Since we are considering the steady state situation, $(A+B)$ is a constant, and hence (8) is easily integrated. Taking the membrane thickness to be δ, we obtain upon integration

$$(A + B) = \frac{2RT}{\delta} (c_i - c_0).$$

Substituting this back into (8) and integrating between 0 and x, we get:

$$c^+ = c^- = c = \frac{(c_i - c_0)}{\delta} x + c_0 \tag{9}$$

giving us the concentration profiles which we sought.

There are two points that should be noted with regard to equation (9). First, since the only restriction in its derivation was the existence of a steady state, it is valid when a finite current flows across the membrane. Second, the concentration profile given in (9) is invariant to current; that is, once it is attained, it will not be distorted by the passage of current through the membrane. This latter result is unique to the single salt case.

From (3c) the complete current voltage characteristic of the membrane can now be obtained. Taking the potential of the outer solution as 0 and that of the inner solution as $-\Psi$, we have upon substitution of (9) into (3c):

$$\Psi = I \int_0^{\delta} \frac{dx}{F(u+v)c} + \frac{RT}{F} \int_{c_0}^{c_i} \frac{(u-v)dc}{(u+v)c}$$

$$= \frac{I}{F(u+v)} \int_0^{\delta} \frac{dx}{\left(\frac{c_i - c_0}{\delta}\right) x + c_0} + \frac{RT}{F} \frac{(u-v)}{(u+v)} \int_{c_0}^{c_i} \frac{dc}{c}$$

$$\Psi = I \frac{\delta}{F} \frac{1}{(u+v)(c_i - c_0)} \ln \frac{c_i}{c_0} + \frac{RT}{F} \frac{(u-v)}{(u+v)} \ln \frac{c_i}{c_0} \tag{10}$$

Equation (10) is the steady state $\Psi - I$ characteristic for the single salt case; a plot of this equation is given in Figure 2. Notice that Ψ is a linear function

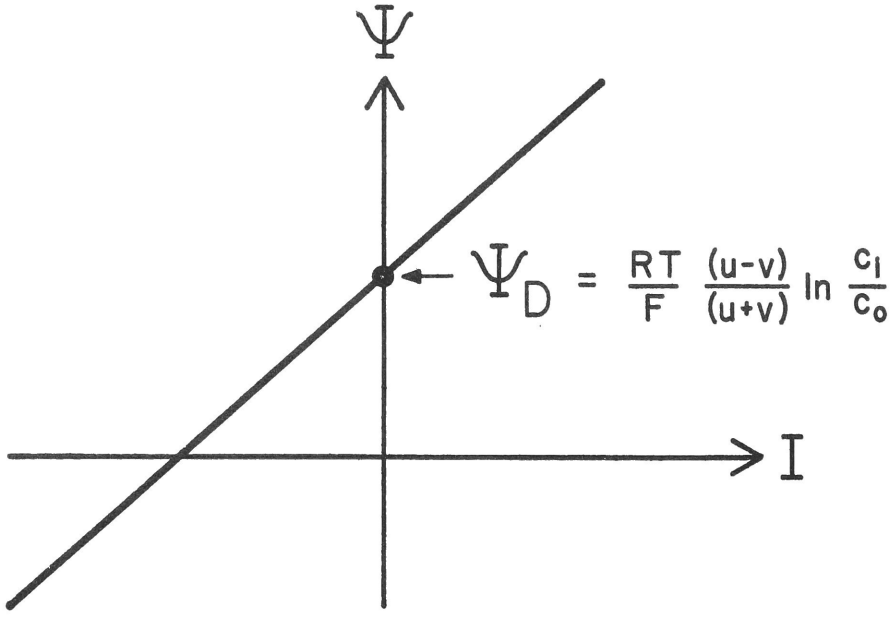

Figure 2. The steady state Ψ -I characteristic for a homogeneous uncharged membrane separating solutions containing the same salt at different concentrations. The membrane behaves as a simple ohmic element except that at I = 0, the transmembrane potential is not zero but is instead Ψ_D , as given by equation (12).

of I, the membrane behaving as a simple ohmic element with resistance, $R_{int.}$, given by:

$$R_{int.} = \frac{\delta}{F} \frac{1}{(u+v)(c_i - c_0)} \ln \frac{c_i}{c_0} \quad . \tag{11}$$

This linear characteristic follows intuitively from the fact that the ionic profiles do not shift in the face of current flow. Added onto the IR contribution to Ψ is the diffusion emf.:

$$\Psi_D = \frac{RT}{F} \frac{(u-v)}{(u+v)} \ln \frac{c_i}{c_0} \tag{12}$$

thus shifting the entire characteristic by this amount above the I-axis (see Figure 2). As we see, Ψ_D is also a constant invariant to current flow, which again is an obvious consequence of the imperturbability of the ionic profiles in the membrane.

2. Equal total concentration of ions on the two sides of the membrane. We choose to consider this case rather than a more general one only because it can be proved that for this situation

$$\frac{d\psi}{dx}$$

is a constant (Appendix I); i.e., there is a "constant field" within the membrane, and hence the solution of the flux equations is greatly facilitated. Furthermore, all of the interesting features of the general case appear in the present instance.

Taking $\psi = 0$ at $x = 0$ and $\psi = -\Psi$ at $x = \delta$, the flux equations become, because of the existence of a constant field within the membrane:

$$\phi_j^+ = - RTu_j \frac{dc_j^+}{dx} - u_j c_j^+ F \frac{d\psi}{dx} = - RTu_j \frac{dc_j^+}{dx} + u_j c_j^+ F \frac{\Psi}{\delta}$$

$$\phi_k^- = - RTv_k \frac{dc_k^-}{dx} + v_k c_k^- F \frac{d\psi}{dx} = - RTv_k \frac{dc_k^-}{dx} - v_k c_k^- F \frac{\Psi}{\delta}$$

Upon integration we obtain:

$$\phi_j^+ = \frac{F u_j \Psi}{\delta} \frac{(c_j^+)_0 - (c_j^+)_i e^{-\frac{F\Psi}{RT}}}{1 - e^{-\frac{F\Psi}{RT}}} \tag{13a}$$

$$\phi_k^- = \frac{F v_k \Psi}{\delta} \frac{(c_k^-)_i - (c_k^-)_0 e^{-\frac{F\Psi}{RT}}}{1 - e^{-\frac{F\Psi}{RT}}} \tag{13b}$$

Summing these equations for all species and remembering that

$$I \equiv \sum_j \phi_j^+ - \sum_k \phi_k^-$$

we have:

$$I = \frac{F\Psi}{\delta} \frac{(U_0 + V_i) - (U_i + V_0) e^{-\frac{F\Psi}{RT}}}{1 - e^{-\frac{F\Psi}{RT}}} \tag{14}$$

[Equations (13) and (14) were obtained by Hodgkin and Katz (1949) in essentially the same manner as employed above. We wish to emphasize, however, that this method is valid only for the case when the total concentration on both sides of the membrane is the same. It is only then that a "constant field" exists within the membrane. In the Hodgkin and Katz paper, the electric field was assumed constant; this will in general not be correct.] Equation (14) gives the complete steady state $\Psi - I$ characteristic for the case when the membrane separates equal total concentrations; a qualitative plot of this relation is given in Figure 3 for the particular case of a membrane separating equal concentrations of KCl and HCl.

We now wish to comment on certain implications of equation (14). The first new feature of interest that arises once we get away from the single salt case is the non-linearity of the steady state $\Psi - I$ characteristic. This is a consequence of the shifting of ionic profiles with the membrane in the face of current flow. (The explicit calculation of the ionic profiles, along with some other relevant quantities, is given in Appendix II.) Because of the non-linear $\Psi - I$ characteristic, a certain ambiguity arises when one speaks of

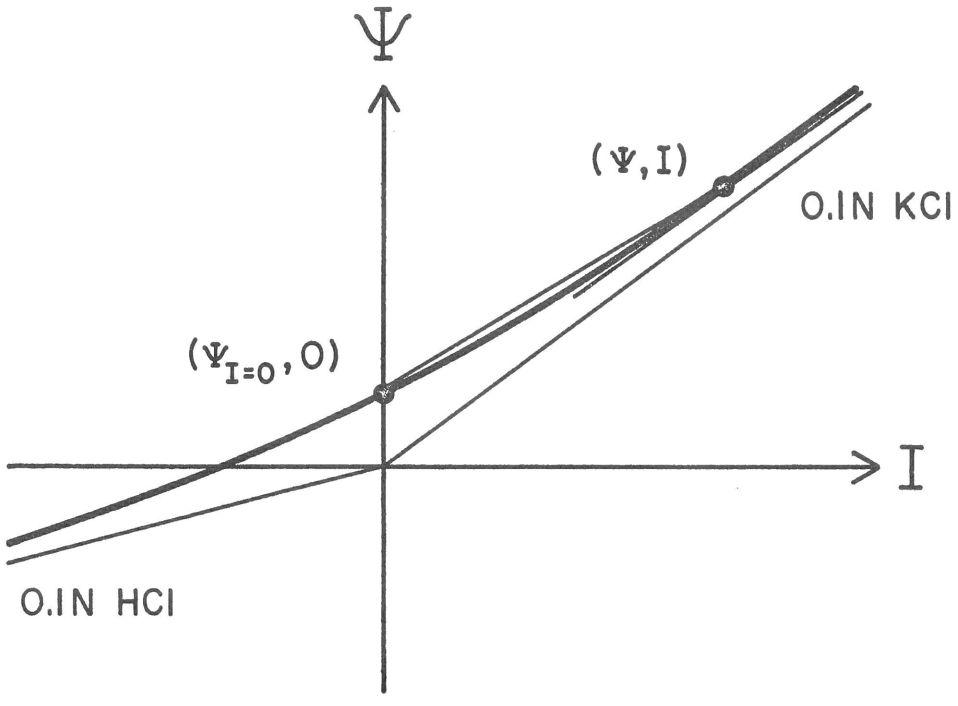

Figure 3. The steady state Ψ -I characteristic for a homogeneous uncharged membrane separating a 0.1 N HCl solution from a 0.1 N KCl solution. The slope of the line drawn between ($\Psi_{I=0}$, 0) and (Ψ , I) is the chord resistance at the potential Ψ; the slope of the tangent at (Ψ, I) is the slope resistance at the potential Ψ . Note that for large positive currents, the Ψ -I characteristic approaches the characteristic for a 0.1 N KCl solution and for large negative currents it approaches the characteristic for a 0.1 N HCl solution. This is a consequence of "flushing" the membrane with 0.1 N KCl for large positive currents and with 0.1 N HCl for large negative currents.

the membrane "resistance". The most unambiguous resistance is the "slope resistance" defined as:

$$\frac{d\psi}{dI}$$

at a given value of Ψ (or I). Operationally, this is obtainable either by determining the entire $\Psi - I$ characteristic and measuring the slope at the desired Ψ, or, if the system has reached a steady state at the desired value of Ψ, by applying a small step of current ΔI and measuring the resulting steady state $\Delta\Psi$; that is,

$$\frac{d\psi}{dI} \approx \left(\frac{\Delta\Psi}{\Delta I}\right)_{t\to\infty}$$

One may also, however, speak of the chord resistance, defined for a given value of Ψ as the slope of the chord drawn between the points $(\Psi_{I=0}, 0)$ and (Ψ, I). In Figure 3, both the slope and chord resistance for a particular point on the $\Psi - I$ characteristic are illustrated.

A second new feature, which although not explicit in (14) is implicit when we consider the passage of the system from a steady state at (Ψ_1, I_1) to a new steady state at (Ψ_2, I_2), is that the system behaves as a time variant resistance. Thus, if the current is "instantaneously" taken from I_1 to I_2, the potential undergoes an "instantaneous"* change to a value Ψ_3 [where clearly

$$(\Psi_1 - \Psi_3) = (I_1 - I_2) \int_0^{\delta} \frac{dx}{F(U+V)} \ ]$$

and then varies continuously in time, approaching the value Ψ_2 in the limit as $t \to \infty$. The basis of this effect is, of course, the fact that it takes time for the ionic profiles to shift from one steady state condition to another. Con-

*Throughout this paper we shall mean by "instantaneous", times so short that the ionic profiles have essentially undergone no changes. We do not include in this term the time involved for ionic atmospheres to alter, which is many orders of magnitude shorter.

sequently, during this time both

$$\int_o^i \frac{dx}{F(U+V)} \quad \text{and} \quad \frac{RT}{F} \int_o^i \frac{d(U-V)}{(U+V)}$$

will be changing and hence producing changes in the value of Ψ, as is evident from equation (3c). In Figure 4, we show two plots of Ψ vs. t for the indicated KCl - HCl system in response to a positive and negative step of current, respectively. (The general shape of these curves is clear heuristically, but their precise shape cannot as yet be explicitly calculated, because it involves the solution of the flux equations for non-steady state situations--an, at present, unsolved problem.)* From the figure, we see that as a consequence of the time variant resistance property, the membrane appears to possess capacitive or inductive properties. [For a good discussion of time-variant resistances, see Mauro (1961).] We may note that for the single salt case, where the ionic profiles remain constant, the time-variant resistance property is not present. Thus, a step of current leads to an "instantaneous" change of the potential to the new steady state value.

The third feature which appears in (14) is that of rectification; i.e.,

$$\Psi(I) \neq -\Psi(-I)$$

The phenomenon of rectification is of particular biological interest, as it plays a prominent role in the electrical properties of excitable membranes--a point to which we shall return later. For our present discussion, it will suffice to point out that rectification in biological systems is quite dramatic, in the squid axon being greater than a hundred to one (Cole 1962). This is far out of line with what can be achieved with the simple Planck junction which we have

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*We have recently obtained in collaboration with H. Cohen and J. Cooley of the IBM Research Laboratories numerical solutions for this problem on an IBM computer. These calculations have shown that for large currents, the potential does not vary in a monotonic fashion as depicted in Figures 4 but instead overshoots (or undershoots) the final steady state value.

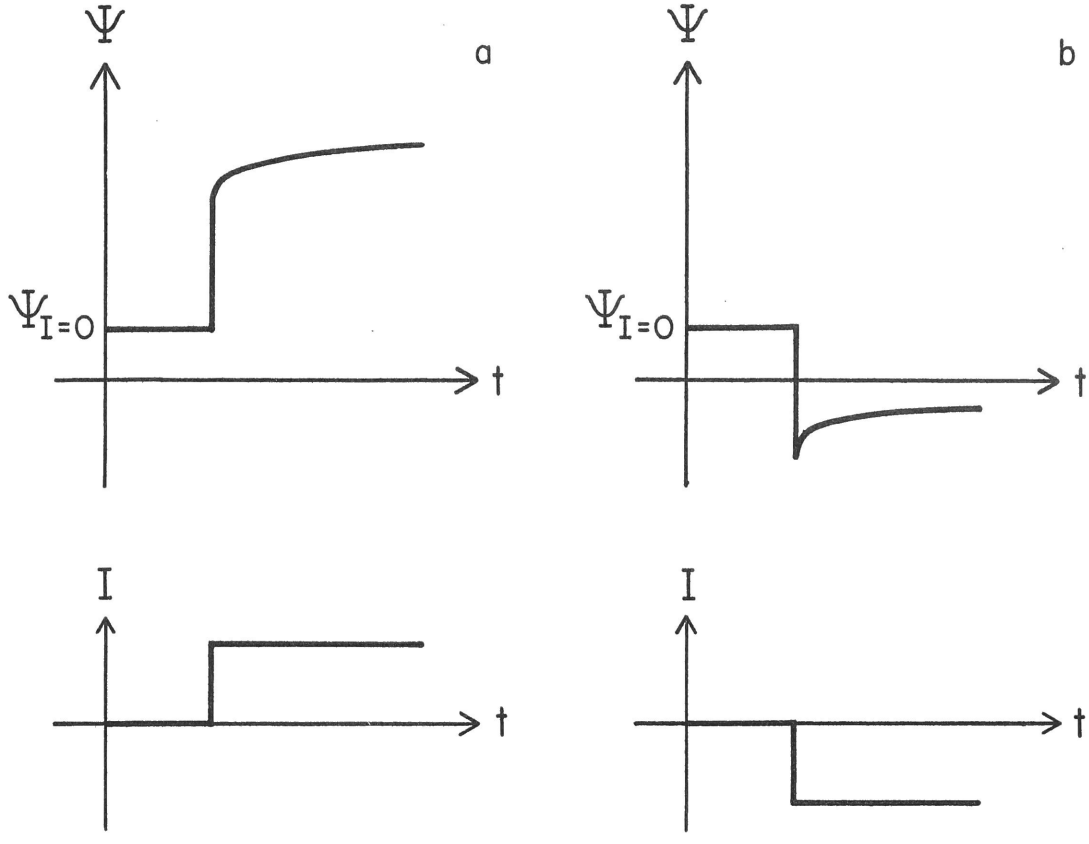

Figure 4. The potential Ψ, as a function of time, in response to a step of current. The system is the same as that described in Figure 3. In 4a the current step is positive, thus causing K^+ (the slower ion) to replace H^+ in the membrane; in 4b the current step is negative, thus causing H^+ to replace K^+ in the membrane. Note that the magnitude of the "instantaneous" change in Ψ upon applying the current is the same in both cases.

been considering. As should be evident from our remarks in this section, rectification in the homogeneous uncharged membrane is due to the movement into or out of the membrane of ions of different mobilities. Thus, in the KCl - HCl system, referred to in Figures 3 and 4, the membrane for large positive currents is virtually "flushed" with the KCl solution, and hence the membrane potential is approximately given by:

$$\left|\Psi\right|_{I\to+\infty} = I\int_0^{\delta} \frac{dx}{F(u_K c_K + u_H c_H + v_{Cl} c_{Cl})} \approx \frac{I}{F(u_K + v_{Cl})} \int_0^{\delta} \frac{dx}{c} =$$

$$= \frac{I\delta}{F(u_K + v_{Cl})c}$$

while for large negative current the membrane is "flushed" with HCl, and we have:

$$\left|\Psi\right|_{I\to-\infty} \approx \frac{I\delta}{F(u_H + v_{Cl})c}$$

Hence, the maximum rectification possible from this particular system is:

$$\frac{\left|\Psi\right|_{I\to+\infty}}{\left|\Psi\right|_{I\to-\infty}} \approx \frac{u_H + v_{Cl}}{u_K + v_{Cl}} \approx 2.5$$

When we realize that the ions chosen in the above example, H^+ and K^+, have a much greater mobility difference than do Na^+ and K^+, the main cationic species on the outside and inside, respectively, of the nerve membrane, it is clear why the "flushing effect" cannot be invoked to explain the large rectification observed in that system, unless one is willing to make drastic assumptions about the mobilities of these ions in the membrane.

This concludes our discussion of the homogeneous-uncharged membrane. We have started our treatment of membrane systems with this particular model because it is the most primitive, basic one--an appreciation of the mechanisms operating in this membrane being prerequisite for an understanding of more complex systems. We have not carried out a complete analysis of this system, but rather have used it to introduce certain ideas and concepts upon which we shall draw in our future discussions. The notation used in this section, and

which will be followed throughout the rest of the paper, is, with some minor changes, that used by Planck in his two famous papers to which we have continually referred. The reader interested in the physics of electrolytic solutions should find the study of these 1890 papers, particularly the first, which includes energy and heat considerations, most interesting and profitable.

C. The Homogeneous-Fixed Charge Membrane

In this section, we shall consider the physical consequences arising from the introduction of a uniform density of fixed-charge into our membrane. This important addition to the homogeneous membrane was originally considered by Teorell (1935) and Meyer and Sievers (Meyer and Sievers, 1936, a, b) In more recent years, Teorell has extended this analysis (Teorell, 1951) and has also given a very comprehensive review of the subject (Teorell, 1953); our treatment of this subject will be based on these latter two papers. The emphasis in the present discussion will be upon the physical assumptions of the fixed-charge theory and their general consequences for the Ψ - I characteristics of the membrane; the reader interested in the detailed solutions of this system for a variety of cases is referred to the two papers of Teorell.

The defining features of the fixed-charge membrane are (a) the presence in the membrane of a uniform density of immobile (fixed), completely ionized charge groups and (b) the assumption that the only interaction between the diffusing ions and the membrane is charge interaction. Both of these assumptions are idealizations which deserve some comment. (The assumptions of the uncharged membrane were, of course, also idealizations.) The concept of a uniform density of charge is clearly unrealizable, since the charges themselves result from ionized groups of finite charge and size. We do not, however, worry about this fine-grain density, but rather consider the "smeared out" average charge density.* If one wishes to have some sort of definite

*The use of continuous charge densities when in reality we are dealing with discrete charges is an approximation used throughout the theory of electrolytes, and in fact the substitution of density functions for discrete systems is prevalent throughout physics in general. The justification of this procedure for each individual case is always performed with a certain amount of hand waving. In the last analysis, the justification comes from the usefulness

physical picture in mind he may imagine the membrane to be a "spaghetti" network (Scatchard, 1955) throughout which fixed charges are randomly distributed, or he may imagine the membrane to be a polyelectrolyte solution (Mauro and Finkelstein, 1958) with the mobility of the polyelectrolyte being very small (zero). The assumption that any properties of the fixed-charge membrane which are different from those of the Planck junction discussed in section B are purely a consequence of the membrane charge deserves emphasis, for it abstracts a very important real membrane property from all other properties and focusses attention on it. Further complications, such as the combination of charge and steric effects [see Sollner (1955)] are a priori excluded.

In order to simplify the analysis of our system, we shall assume, as we did for the uncharged membrane, that the membrane is freely permeable to solvent, that the mobilities of the diffusing ions are not concentration dependent, that their activity coefficients can be taken as 1 both in free solution and within the membrane, and that hydrostatic pressure terms and mass flow of solvent can be neglected. [For a clear analysis of the mass movement of solvent in fixed-charge membranes arising as a consequence of both electro-osmotic and hydrostatic pressure effects, see Schlögl (1955).]

1. The fixed-charge membrane at thermodynamic equilibrium. To begin our treatment of the fixed-charge membrane, let us start with the equilibrium situation arising when a membrane with a concentration, N^+, of positive fixed-charge separates equal concentrations of a single univalent electrolyte. [The same results, with appropriate sign changes, will hold for a negative fixed-charge membrane.] For the uncharged membrane, the concentration of cation and anion within the membrane would be identical

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*(continued) of the resulting theory. We may note that our use of the concept of ionic "concentrations" in the flux equations already has implicit in it a certain kind of averaging. We may finally point out that when attempting to extend the analysis of "thick" membranes to the 100 Å plasma membrane, one must carefully consider whether such macroscopic terms as "concentration" have any real meaning.

with their concentrations in the bathing solutions; it is obvious, however, that such will not be the case in the present instance. On account of the positive charges associated with the membrane, there will result an enhancement of anion concentration and concomitant diminution of cation concentration in the membrane. The two equations governing the equilibrium ionic profiles are the Boltzmann distribution:

$$c^+ = c_0 e^{-\frac{F\psi}{RT}} \tag{15a}$$

$$c^- = c_0 e^{\frac{F\psi}{RT}} \tag{15b}$$

and Poisson's equation:

$$\frac{d^2\psi}{dx^2} = -\frac{4\pi}{\epsilon}\rho \tag{16}$$

where c_0 is the concentration of cation and anion at $\pm\infty$, $\rho = F(c^+ - c^-)$ in free solution, and $\rho = F(N^+ + c^+ - c^-)$ within the membrane.* [It is interesting to note that if ϕ_j in the flux equations (1) is set equal to 0, c_j satisfies the Boltzmann distribution. Thus, at thermodynamic equilibrium, when the fluxes of all ions are 0, the flux equations reduce to the Boltzmann distribution.] Defining the potential ψ at $\pm\infty$ to be 0, and $c^+ = c^- = c_0$ at $\pm\infty$, and assuming the membrane thickness to be much greater than that of the space charge regions, equations (15) and (16) can be solved for c^+, c^-, and ψ as functions of x. A qualitative plot of these functions is given in

*We shall not go into the troublesome question of defending the admissibility of using the same ψ function in both Poisson's equation and the Boltzmann distribution, referring the reader to some pertinent references (Onsager, 1933; Kirkwood, 1934).

Figure 5.* We see that in the bulk of the solution and membrane electroneutrality holds, while in the regions of the membrane solution interfaces, where the cation and anion concentrations are changing from their values in solution to their values in the interior of the membrane, there occur space charge regions throughout which the potential changes. The extent of the space charge regions is determined by the "Debye length" (Debye and Huckel, 1923) of the solution, which for a 0.1 N aqueous solution at room temperature is approximately 10 Å. We see, therefore, that the membrane can be divided into three regions: the two space charge regions and the electroneutral region.

Let us now calculate the potential difference between the bulk solution (away from the diffuse double layer) and the electroneutral region of the membrane. While we could obtain this value by solving the Poisson-Boltzmann equation, this will not be necessary; for clearly, between the solution and the interior of the membrane there exists a Donnan equilibrium. [The space charge analysis demonstrates how this is established. The Donnan equilibrium can be thermodynamically derived, however, simply by equating the electrochemical potential of the cation (or anion) in solution with its value in the membrane (Overbeek, 1956).] We thus have the well known Donnan condition:

$$r \equiv \frac{(c^-)_m}{c_0} = \frac{c_0}{(c^+)_m} \tag{17}$$

where the subscript m denotes the concentration of the appropriate ion in the electroneutral region of the membrane. The Donnan emf., designated

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*The solution of the Poisson-Boltzmann equation for a fixed-charge membrane bathed on one side by an electrolytic solution, both of "infinite" extent, is given by Mauro (Mauro, 1962). If we assume that the extent of the membrane is large compared to the thickness of the double-layer regions, then essentially the same solution will be valid for our finite membrane separating identical solutions of infinite extent. We may mention that the calculation of the profiles for the case where the membrane thickness cannot be taken as infinite is a more formidable algebraic task.

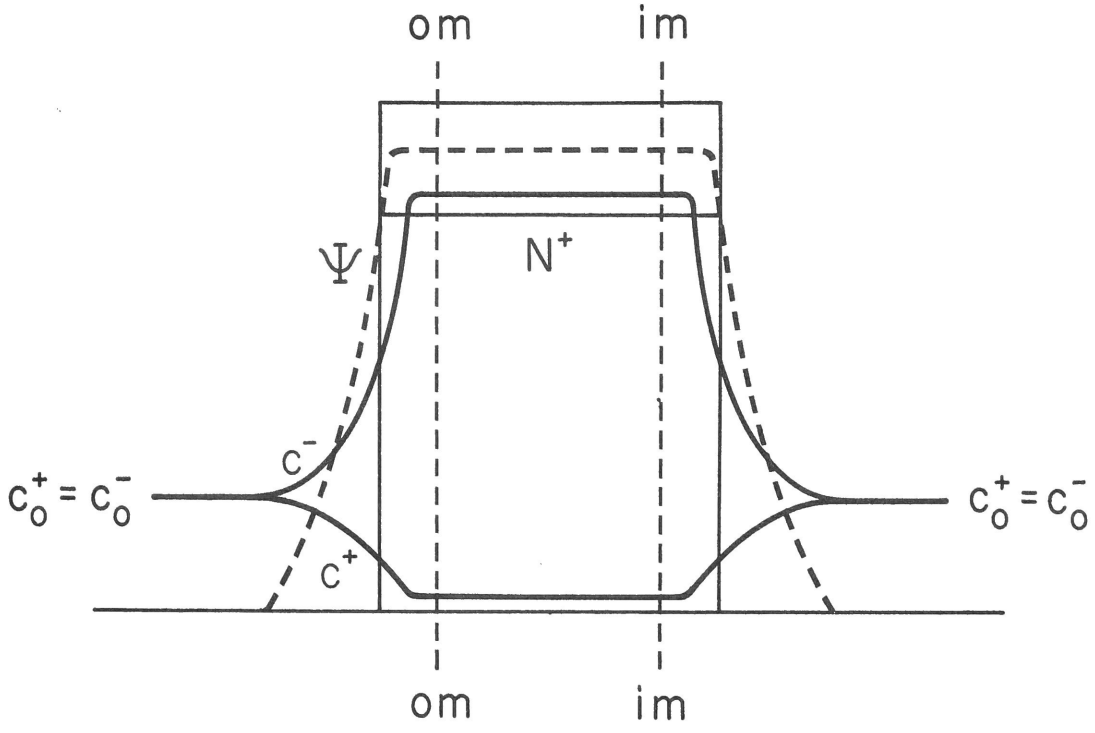

Figure 5. A qualitative plot of the equilibrium concentration and potential profiles arising when a homogeneous fixed-charge membrane of charge density N^+ is bathed on both sides by an electrolyte solution of concentration c_o. The lines om and im mark the boundaries of the electroneutral region in the membrane. The extent of the space charge regions has been highly exaggerated.

by the letter, π, is then given by:

$$\pi = \frac{RT}{F} \ln r = \frac{RT}{F} \ln \frac{(c^-)_m}{(c_0)} \tag{18}$$

Substituting the condition of the electroneutral region:

$$N^+ + (c^+)_m = (c^-)_m \tag{19}$$

into (17), we have:

$$(c^-)_m = \frac{(N^+) + \sqrt{(N^+)^2 + 4c_0^2}}{2} \tag{20}$$

and this may be inserted into (18) to obtain an expression for π in terms of (N^+) and c_0. We see then in Figure 5 that at thermodynamic equilibrium there exists a Donnan equilibrium at each membrane interface, with a concomitant Donnan emf.; the total transmembrane potential is, of course, zero, and we now see that this is a consequence of the algebraic sum of the Donnan emfs. being zero.

Continuing our discussion of the equilibrium situation, let us see what are the consequences of the fixed charges on the membrane conductance. In the uncharged membrane, the concentration of salt will be the same within the membrane as in the surrounding solution [equation (20) reduces to this case when $(N^+) = 0$], so that the specific resistivity of the membrane will be that of the solution. For the charged membrane, however, the concentration of the gegen ion in the membrane is enhanced [equation (20)], so that certainly when (N^+) becomes reasonably large, the membrane conductance will be considerably higher than that of the bathing solution. In fact, for $(N^+) >> c_0$ we see from (20) that

$$(c^-)_m \approx (N^+)$$

and hence the conductance of the membrane will be quite high and virtually invariant to the ionic concentration in the surrounding solution. [We are,

of course, assuming that the double-layer regions are so narrow that their contribution to the IR drop is negligible. The possibility of polarization of the double-layers will be discussed below.] We may further note that as a consequence of the enhancement of gegen ion concentration and simultaneous exclusion of neben ions, the current is carried in the membrane almost exclusively by the gegen ions, when (N^+) is large; i.e. the transference number of the gegen ions is 1.

2. The fixed-charge membrane during the steady state flux of ions. We now wish to extend our treatment of the fixed-charge membrane to non-equilibrium situations. Again restricting our discussion to the single salt case, we must investigate the effect on the ionic profiles of a gradient of salt concentration across the membrane and/or of the passage of current through the membrane. The assumption made by Teorell (1953) is that even when there is a net flux of matter across the membrane, there still exist Donnan equilibria at the two membrane solution interfaces. This, of course, cannot be strictly true, for if there were true equilibrium at the interfaces there would be no matter flux across the membrane. The question, therefore, is to what extent is this approximation valid? [We may mention that the assumption of "local heterogeneous equilibrium at the boundaries of the membrane with the exterior phases..." is not unique to fixed-charge membranes, but is an assumption introduced generally in the treatment of membrane transport (Kirkwood, 1954). Formally, the statement is made that:

$$(\bar{\mu}_j)_0 = (\bar{\mu}_j)_{0m}$$

$$(\bar{\mu}_j)_i = (\bar{\mu}_j)_{im}$$

where $\bar{\mu}_j$ is the electrochemical potential of the j'th species and the subscripts $0m$ and im refer to its value "just inside" the membrane at the outer and inner interfaces, respectively; for the fixed-charge membrane, $(\bar{\mu}_j)_{0m}$ and $(\bar{\mu}_j)_{im}$ would refer to the values of the electrochemical potential at the beginning of the electroneutral regions (see Figure 5).]

Considering first the free diffusion case (I = 0) where the membrane separates different concentrations of electrolyte, we may view the kinetics of the diffusion process as follows: at each boundary the ions are striving, as a consequence of their thermal motion, to satisfy the Donnan equilibrium, but the consequent gradient of concentration results in a diffusion process which tends to upset these conditions. From this picture we can readily appreciate the reasonableness of the Teorell approximation. So long as the rate of diffusion through the membrane is slow compared to the kinetics of the boundary processes, there will be little perturbation of the equilibrium boundary conditions by the net flux of matter, and hence the Donnan conditions will be satisfied at each interface. Because of the narrowness of the diffuse double-layer regions compared to the total membrane thickness, it follows, from the above remarks, that unless there is a huge difference in concentration between the inner and outer solutions, there will exist essentially Donnan equilibria at the interfaces. We see, however, that for very thin membranes (e.g. the plasma membrane) the rate of transmembrane diffusion, because of the now steep concentration gradients, may become comparable to the diffusion rates at the interfaces, and the assumption of equilibria at the membrane solution boundaries will no longer be valid. The justification of the claim that the boundary equilibria will not be significantly perturbed by current flow is similar to the argument given above for free diffusion under a concentration gradient. [As for the case of very rapid diffusion (steep concentration gradients) it is understood that for very large current densities, the double layers will be perturbed.]

With the assumption of Donnan equilibria always existing at the boundaries, the analysis of the fixed-charge membrane becomes quite straightforward. Taking the concentrations of electrolyte just after the Donnan "jumps" as the boundary conditions, we have within the electroneutral region of the membrane a Planck diffusion regime which can be treated by the same general method as employed in the homogeneous uncharged membrane.* [The

*We see that in this approach the detailed structure of the double-layer regions does not enter into the arguments, nor is it needed.

algebra is a little more difficult, because of the presence of the fixed charges, but there are no new physical difficulties involved (see Teorell (1951) for the general integration of the flux equations).] It must be remembered that because of the fixed charges, the condition of electroneutrality will not be given by (4) but rather by:

$$\sum_j c_j^+ + \omega \bar{X} = \sum_k c_k^- \tag{21}$$

where $\bar{X}$ denotes the concentration of fixed charge and ω is the sign of the charge (+1 or -1).* The total membrane potential will be given by the algebraic sum of the two Donnan emfs. plus the Planck diffusion emf. in the electroneutral region.

We could now go on and consider the electrical and flux properties for various cases of a fixed-charge membrane separating different electrolytic solutions, but as this has been done rather comprehensively by Teorell (1953), the interested reader is referred to that source. In order to illustrate the handling of the flux equations for a fixed-charge membrane, however, we conclude this section with the derivation of a general result which we shall need for our future calculations.

Let us denote by c_j the concentration of the j'th ion at any point x within the electroneutral region of the membrane. Then we have for the flux equations:

$$A_j \equiv -\frac{\phi_j^+}{u_j} = RT\frac{dc_j^+}{dx} + Fc_j^+\frac{d\psi}{dx}$$

$$B_k \equiv -\frac{\phi_k^-}{v_k} = RT\frac{dc_k^-}{dx} - Fc_k^-\frac{d\psi}{dx}$$

Summing these equations for all species, we have:

$$(A + B) = RT\frac{d}{dx}\left(\sum_j c_j^+ + \sum_k c_k^-\right) + F\frac{d\psi}{dx}\left(\sum_j c_j^+ - \sum_k c_k^-\right)$$

where, by definition:

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*$\bar{X}$ has the same meaning as (N) used previously.

$$A \equiv \sum_j A_j \equiv - \sum_j \frac{\phi_j^+}{u_j}$$

$$B \equiv \sum_k B_k \equiv - \sum_k \frac{\phi_k^-}{u_k}$$

Applying the electroneutrality condition [equation (21)] we obtain:

$$(A + B) = RT \frac{d}{dx} (2 \sum_j c_j^+ + \omega \bar{X}) - F\omega \bar{X} \frac{d\psi}{dx} = 2RT \frac{dc^+}{dx} - F\omega \bar{X} \frac{d\psi}{dx}$$

where,

$$c^+ \equiv \sum_j c_j^+$$

Since we are restricting our discussion to the steady state, (A + B) is a constant throughout the membrane, and the above equation can be integrated between the point 1m (the point in the electroneutral region of the membrane just after the first Donnan "jump") and any other point x within the electroneutral region to give:

$$c_x^+ = c_{1m}^+ + 0.5\, \omega \bar{X} \frac{F}{RT} \psi_x + \frac{(A+B)}{2RT} x \qquad (22)$$

D. The Two Layer Sandwich Membrane

We are now in a position to derive and discuss the steady state current voltage relation of a sandwich membrane consisting of a positive and negative fixed-charge membrane juxtaposed in series (Figure 6). We shall find that in the face of a constant current, the total membrane potential will be the sum of two terms: one, the IR-drop in the electroneutral regions, and two, the emfs. in the space charge regions. Our task is to obtain expressions for these terms and to assess their relative importance. The analysis is greatly facilitated if the system is symmetrical. For this reason, we shall take the thickness and charge densities of the positive and negative fixed-charge regions to be identical and shall take for our solution on side 1 and side 2, equal concentrations, c_0, of KCl, where we assume that $u_K = v_{Cl} \equiv u$. [By convention, we shall

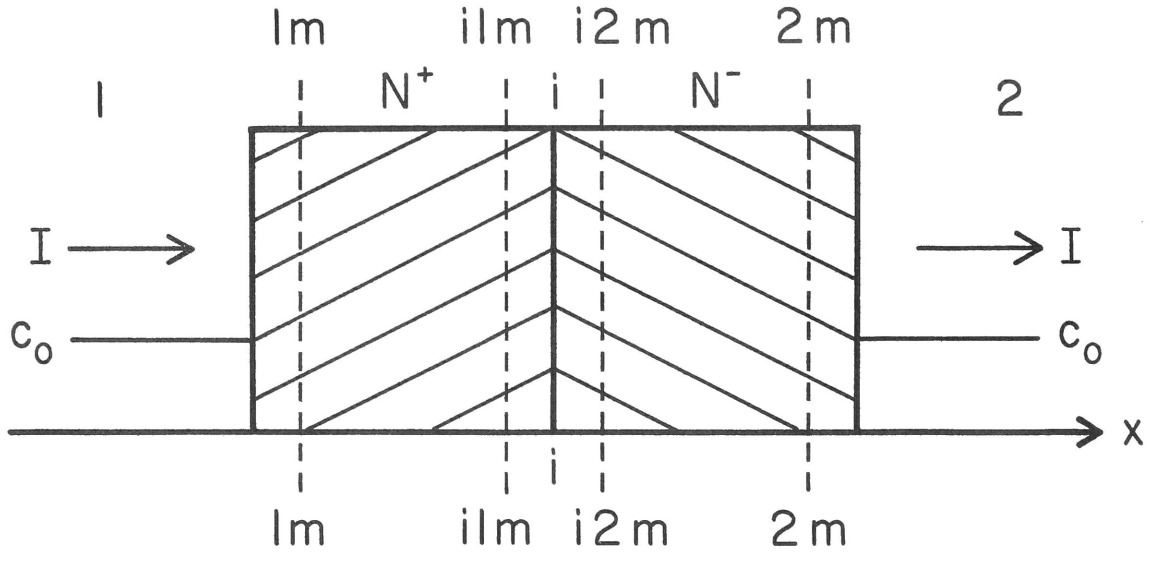

Figure 6. A sandwich membrane consisting of a positive and negative fixed-charge membrane of equal charge densities in series. The region between 1m and i1m and the region between i2m and 2m are the electroneutral regions in the positive and negative fixed-charge lattices, respectively. The direction of positive current flow is indicated by the arrows.

mean by positive current flow that current is flowing from the positively charged to the negatively charged region.]*

We begin by deriving a general equation arising from the symmetry properties of our system. By definition:

$$I = \phi_K - \phi_{Cl}$$

and from symmetry considerations it is clear that:

$$\phi_K = - \phi_{Cl} = \frac{1}{2} I \tag{23}$$

Integrating the flux equation

$$\phi_K = - u(RT \frac{dc_K}{dx} + Fc_K \frac{d\psi}{dx})$$

from 1 to i, the center of the sandwich, and from i to 2, we obtain the two equations:

$$\phi_K \frac{1}{Fu} \int_1^i \frac{dx}{c_K} = - \frac{RT}{F} \ln \frac{c_i}{c_0} - (\psi_i - \psi_1) \tag{24a}$$

$$\phi_K \frac{1}{Fu} \int_i^2 \frac{dx}{c_K} = - \frac{RT}{F} \ln \frac{c_0}{c_i} - (\psi_2 - \psi_i) \tag{24b}$$

Again, from symmetry, we have that

$$(\psi_i - \psi_1) = (\psi_2 - \psi_i)$$

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*For those familiar with the solid state literature a comparison is given in Appendix III between the analysis in this section and the analysis of the p-n junction.

and hence on subtracting (24b) from (24a) we obtain:

$$2\frac{RT}{F}\ln\frac{c_0}{c_i} = \frac{\phi_K}{Fu}\left(\int_1^i \frac{dx}{c_K} - \int_i^2 \frac{dx}{c_K}\right), \tag{25}$$

Instead of integrating (24a) and (24b) from 1 to i and from i to 2, we could have integrated from 1m to i1m and from i2m to 2m, where the subscript m refers to points in the membrane (see Figure 6). By choosing these points so as to maintain symmetry, that is:

$$\begin{aligned}(1 - 1m) &= (2m - 2)\\ (i1m - i) &= (i - i2m)\end{aligned} \tag{26}$$

then again the potential terms will subtract out, and we get:

$$\frac{RT}{F}\ln\frac{(c_K)_{1m}(c_K)_{2m}}{(c_K)_{i1m}(c_K)_{i2m}} = \frac{\phi_K}{Fu}\left(\int_{1m}^{i1m}\frac{dx}{c_K} - \int_{i2m}^{2m}\frac{dx}{c_K}\right), \tag{25a}$$

We note that equation (25) follows from the flux equations and symmetry considerations, and is <u>not</u> dependent on any "electroneutrality" assumptions. Because of its general validity, it will serve as a standard for testing the admissibility of some approximations which we shall have to make.

We turn now to a calculation of the contribution of the electroneutral region to the total membrane potential; toward this end, we must determine the ionic profiles within the membrane. Confining our attention to the positive fixed-charge lattice, let us obtain the profiles between 1m and i1m, where these points are far enough in the membrane that electroneutrality can be assumed to hold between them. Within this region, equation (22) is valid; furthermore, from (23) we see that $(A+B) = 0$. Thus (22) becomes:

$$c_K = (c_K)_{1m} + 0.5\,\omega\bar{X}\cdot\frac{F}{RT}\psi_x \tag{27}$$

Since $u_K = v_{Cl}$, there is no diffusion emf. in the electroneutral region, so

that ψ_x must be a consequence of IR-drop. That is,

$$\psi_x = - I \int_{1m}^{x} \frac{dx}{F(U+V)} = - \frac{I}{Fu} \int_{1m}^{x} \frac{dx}{c_K + c_{Cl}} = - \frac{I}{Fu} \int_{1m}^{x} \frac{dx}{2c_K + \omega \bar{X}} \tag{28}$$

Substituting this into (27) and then differentiating with respect to x yields:

$$2RT \frac{dc_K}{dx} = - \frac{I\omega \bar{X}}{u} \cdot \frac{1}{2c_K + \omega \bar{X}}$$

Taking $x = 0$ at the point 1m, we obtain for the solution of this equation:

$$c_K = \frac{- \omega \bar{X} + \sqrt{(\omega \bar{X})^2 + 4ax + 4b}}{2} \tag{29a}$$

where,

$$a = - \frac{I\omega \bar{X}}{2RTu} \tag{30a}$$

$$b = (c_K^+)_{1m}^2 + \omega \bar{X} (c_K^+)_{1m} \tag{30b}$$

and from the condition of electroneutrality we have:

$$c_{Cl} = \frac{\omega \bar{X} + \sqrt{(\omega \bar{X})^2 + 4ax + 4b}}{2} . \tag{29b}$$

Equations (29) give the ionic profiles in the electroneutral region of the positive fixed-charge lattice; analogous expressions will hold in the negatively charged lattice. Substituting (29a) into (28) and integrating between 1m and ilm we obtain for the potential difference (IR-drop) in the electroneutral region of the positive fixed-charge lattice:

$$(\psi_{ilm} - \psi_{1m}) = \frac{RT}{F} \frac{2}{\omega \bar{X}} [(c_K^+)_{ilm} - (c_K^+)_{1m}] . \tag{31}$$

For the total IR-drop, $\psi_{int.}$, in the electroneutral regions we have from

$$\psi_{int.} = 2(\psi_{ilm} - \psi_{lm}).$$

Calling the thickness of the electroneutral region δ, we then have on substituting (29a) into (31):

$$\psi_{int.} = 2\frac{RT}{F}\frac{1}{\omega\bar{X}}\left[\sqrt{(\omega\bar{X})^2 + 4a\delta + 4b} - \sqrt{(\omega\bar{X})^2 + 4b}\right] \qquad (32)$$

Having calculated the contribution of the electroneutral regions to the total membrane potential, we now wish to do the same for the space charge regions. Assuming the space charge regions are very thin compared to the total membrane thickness, we may neglect the IR-drops occurring within them. It therefore remains to determine the diffusion emf. in these regions. By the same arguments as we used for the single fixed-charge membrane, we shall assume that Donnan equilibria exist at the four interfaces: 1 - 1m; ilm - i; i - i2m; and 2m - 2.*

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*It is interesting to compare the equation obtained from this assumption with the exact relation given in (25). From the Donnan assumption and symmetry we have:

$$r_{lm} = \frac{(c_K^+)_{lm}}{c_0} = \frac{1}{r_{2m}} = \frac{c_0}{(c_K^+)_{2m}}$$

$$r_{il} = \frac{(c_K^+)_{ilm}}{c_0} = \frac{1}{r_{i2}} = \frac{c_i}{(c_K^+)_{i2m}}$$

Substituting these equations into (25a) we obtain:

$$2\frac{RT}{F}\ln\frac{c_0}{c_i} = \frac{\phi_K}{Fu}\left(\int_{lm}^{ilm}\frac{dx}{c_K} - \int_{i2m}^{2m}\frac{dx}{c_K}\right). \qquad (25)'$$

Comparing this with (25) we see that it is in agreement with that equation provided that the following inequality holds:

$$\left(\int_{l}^{lm}\frac{dx}{c_K} + \int_{ilm}^{i}\frac{dx}{c_K} - \int_{i}^{i2m}\frac{dx}{c_K} - \int_{2m}^{2}\frac{dx}{c_K}\right) << \left(\int_{lm}^{ilm}\frac{dx}{c_K} - \int_{i2m}^{2m}\frac{dx}{c_K}\right).$$

But this is equivalent to our assumption that the space charge regions are very thin compared to the electroneutral regions.

We then have for the Donnan emfs.:

$$\pi_{1m} = \frac{RT}{F} \ln \frac{(c^+_K)_{1m}}{c_0}$$

$$\pi_{ilm} = \frac{RT}{F} \ln \frac{(c^+_K)_{ilm}}{c_0}$$

and hence, by symmetry, the total transmembrane Donnan emf., π, is:

$$\pi = 2(\pi_{ilm} - \pi_{1m}) = \frac{2RT}{F} \left(\ln \frac{(c^+_K)_{ilm}}{c_i} - \ln \frac{(c^+_K)_{1m}}{c_0} \right) . \qquad (33)$$

From the Donnan conditions:

$$\frac{(c^+_K)_{ilm}}{c_i} = \frac{c_i}{(c^-_{Cl})_{ilm}} ; \qquad \frac{(c^+_K)_{1m}}{c_0} = \frac{c_0}{(c^-_{Cl})_{1m}}$$

we obtain using (29a) and (29b):

$$c_i = \sqrt{a\delta + b} \qquad (34)$$

$$c_0^2 = b \qquad (35)$$

Thus, (33) becomes:

$$\pi = \frac{2RT}{F} \ln \frac{c_0(-\omega\bar{X} + \sqrt{(\omega\bar{X})^2 + 4a\delta + 4c_0^2})}{(\sqrt{a\delta + c_0^2})(-\omega\bar{X} + \sqrt{(\omega\bar{X})^2 + 4c_0^2})} . \qquad (36)$$

The sum of (32) and (36) gives the complete Ψ-I characteristic of the membrane. It is interesting to compare the relative magnitude of these two contributions to the total membrane slope resistance at I = 0. We have from (32):

$$\frac{d\psi_{int.}}{dI} = -\frac{2\delta}{Fu} \frac{1}{\sqrt{(\omega\bar{X})^2 + 4a\delta + 4b}} \qquad (37)$$

While from (36) we get:

$$\frac{d\pi}{dI} = -\frac{\delta(\omega\bar{X})^2}{2Fu} \frac{1}{(a\delta + b)\sqrt{(\omega\bar{X})^2 + 4a\delta + 4b}} \qquad (38)$$

Equations (37) and (38) give the contributions to the slope resistance (as a function of I) of the electroneutral regions and Donnan regions respectively. Passing to the limit of $I \to 0$ we obtain:

$$\left(\frac{d\psi_{int.}}{dI}\right)_{I=0} = -\frac{2\delta}{Fu}\,\frac{1}{\sqrt{(\omega\bar{X})^2 + 4c_0^2}} \tag{37a}$$

$$\left(\frac{d\pi}{dI}\right)_{I=0} = -\frac{\delta}{2Fu}\,\frac{(\omega\bar{X})^2}{c_0^2}\,\frac{1}{\sqrt{(\omega\bar{X})^2 + 4c_0^2}} \tag{38a}$$

We see that as $\omega\bar{X} \to \infty$,

$$\left(\frac{d\psi_{int.}}{dI}\right)_{I=0} \to 0 \quad \text{while} \quad \left(\frac{d\pi}{dI}\right)_{I=0} \to \infty .$$

Thus, for highly fixed charged membranes, virtually all of the "resistance" is due to the emf. generated in the central space charge region, while the IR-drop across the membrane becomes trivial. These conclusions may be seen intuitively from the following considerations. For large fixed-charge densities, the conductance of the electroneutral regions becomes quite high because of the large gegen ion concentration. On the other hand, the concentration c_i at the center of the junction will have to be very much different from c_0, even for small currents, for a steady state to be achieved, and consequently a large Donnan emf. is produced. The plot of π vs. I [equation (36)] is given in Figure 7 for a moderate fixed-charge density. We see that this system shows marked rectification. The basis for this is most easily understood by considering equation (34) which can be rewritten from (30a) and (35) as:

$$c_i = \sqrt{c_0^2 - \frac{I\omega\bar{X}}{2RTu}} .$$

For positive currents approaching $\frac{2RTu}{\omega\bar{X}}$, c_i approaches 0 and hence the resulting Donnan emf. becomes infinite; for equal negative current, c_i remains finite and the Donnan emf. is clearly well behaved. [We may note that as $I \to +\frac{2RTu}{\omega\bar{X}}$ our entire analysis breaks down, since all our assumptions

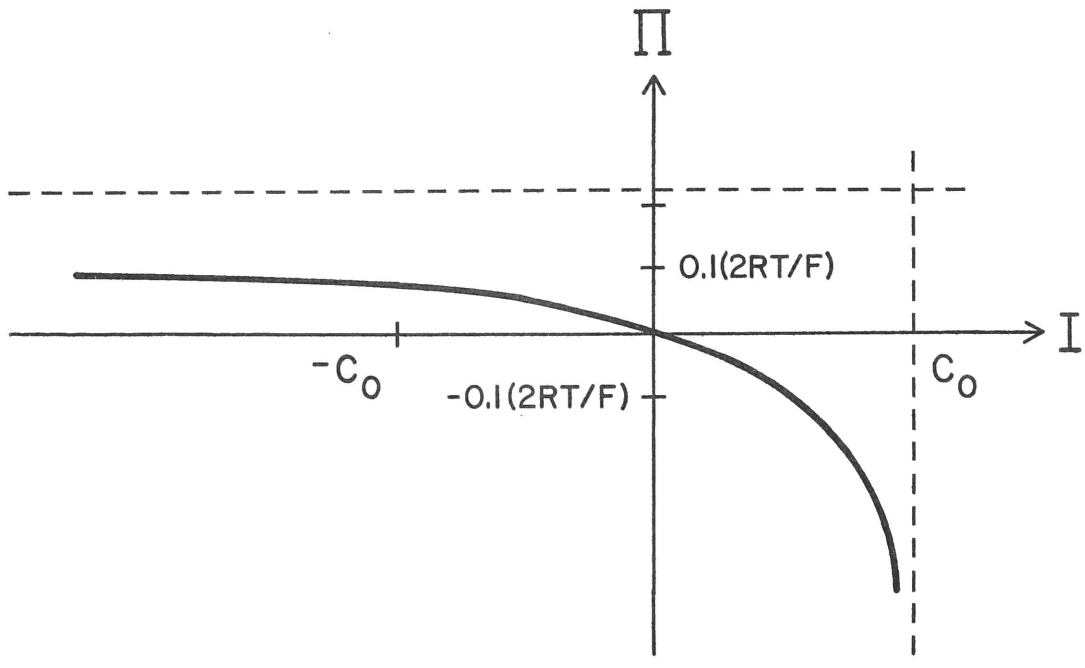

Figure 7. A plot of equation (36) for $\omega \bar{X} = c_o$ where we have taken $\delta / 2RTu = 1$.

about the space charge regions being small compared to the electroneutral regions will no longer be valid. Furthermore, we can no longer neglect the effects of H^+ and OH^- when c_i reaches values around 10^{-7} moles/liter. The rectification becomes dramatic, however, before these theoretical limitations are reached.]

The fact that the membrane "resistance" for the sandwich membrane is primarily the result of an emf. generated in the face of current flow deserves emphasis and should be compared with the homogeneously charged and uncharged membranes. In these latter systems, the resistance is mainly ohmic (although there is in general some polarization resulting from the alteration of the ionic profiles by the impressed current) and hence, if the current flowing through such membranes is suddenly interrupted, there will be an "instantaneous" drop in transmembrane potential to approximately zero, this drop in potential representing the IR contribution to the total membrane potential. On the contrary, if the current is interrupted in the sandwich membrane, the potential seen "instantaneously" across the membrane will be essentially the same as that existing during the current flow, because of the conservative nature of this potential, i.e. the Donnan emfs. As a result of diffusion, c_i will change with time and eventually reach c_0. During this time, the membrane potential will decay in an exponential manner back to zero.

Experimental: Experiments have been carried out using for the sandwich membrane bipolar AMFion membranes whose thickness is $\sim$ 300 μ. One half of the membrane contains positive fixed charges (quaternary nitrogen), and the other negative fixed-charge groups (sulphonic acid). The matrix of both halves is polyethylene, and the junction of the two is formed by thermoplastically sealing them together in a carver press. In our experiments, the membrane was clamped in a two-compartment lucite chamber of approximately 1 sq. cm. cross sectional area, each compartment being filled with identical solutions of KCl (Figure 8). A pair of Ag/AgCl electrodes were used for passing steps of current (obtained from an A. E. L. stimulator

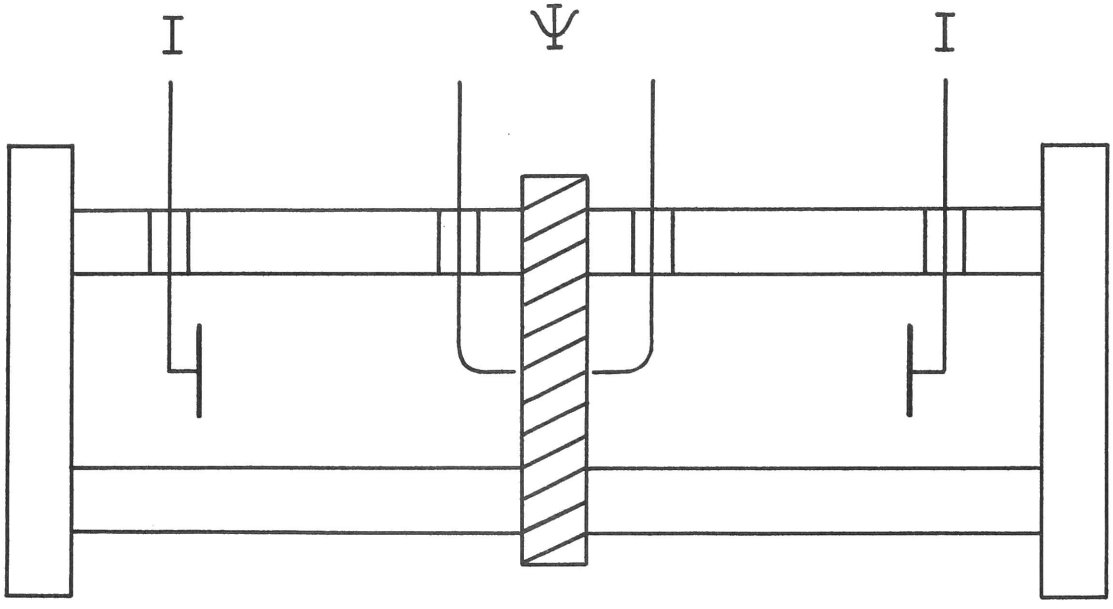

Figure 8. Schematic sketch of the lucite chamber used for obtaining records both on synthetic membranes and on frog skin and toad bladder. The cross sectional area of the chamber is one square centimeter.

with a megohm resistance in series) across the membrane, and the resulting voltage response was measured by a pair of saturated calomel electrodes coupled to the two surfaces of the membrane through saturated Agar-KCl bridges. [The calomel electrodes were connected to a Tektronix 502 dual beam oscilloscope with an input impedance of 10 megohms.]

In Figure 9, we see a series of records obtained with 0.1 N KCl on the two sides of the membrane.* Let us note several features in qualitative agreement with the theory presented in the previous paragraphs. First, we see from our measurements with small currents (Figure 9a) that the membrane resistance is quite large, being around 2.5 KΩ. The resistances of the individual fixed-charge halves of the membrane were found in separate experiments to be approximately 30Ω. Thus, large resistance is not a property of the individual halves of the membrane but results from the coupling of the two. Second, we observe in the same record that the potential does not drop immediately upon interruption of the current, but decays exponentially with time. (The system behaves like a parallel RC network.) We see

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*It should be understood that because of the thickness of these membranes the duration of the current stimulus (1 second) is much too short for a steady state to have been reached; in other experiments, it was found that it required several hours for attainment of a steady state condition. Thus, while the concentration of KCl in and near the center of the junction is significantly perturbed during the 1-second pulse, the concentration profiles within the bulk of the membrane have not had time to be appreciably altered. These experiments, however, are presented only to illustrate the physical principles discussed in this section and to indicate the magnitude of the effects which can be obtained. The fact that large rectification and "resistance" is observed in so short a period of time (when there has been little change in the bulk of the membrane) lends added interest to these systems in that it indicates that even for thin membranes (i.e., the plasma membrane) in which there are sudden changes in charge density and sign, the analysis which we have given can have at least qualitative significance.

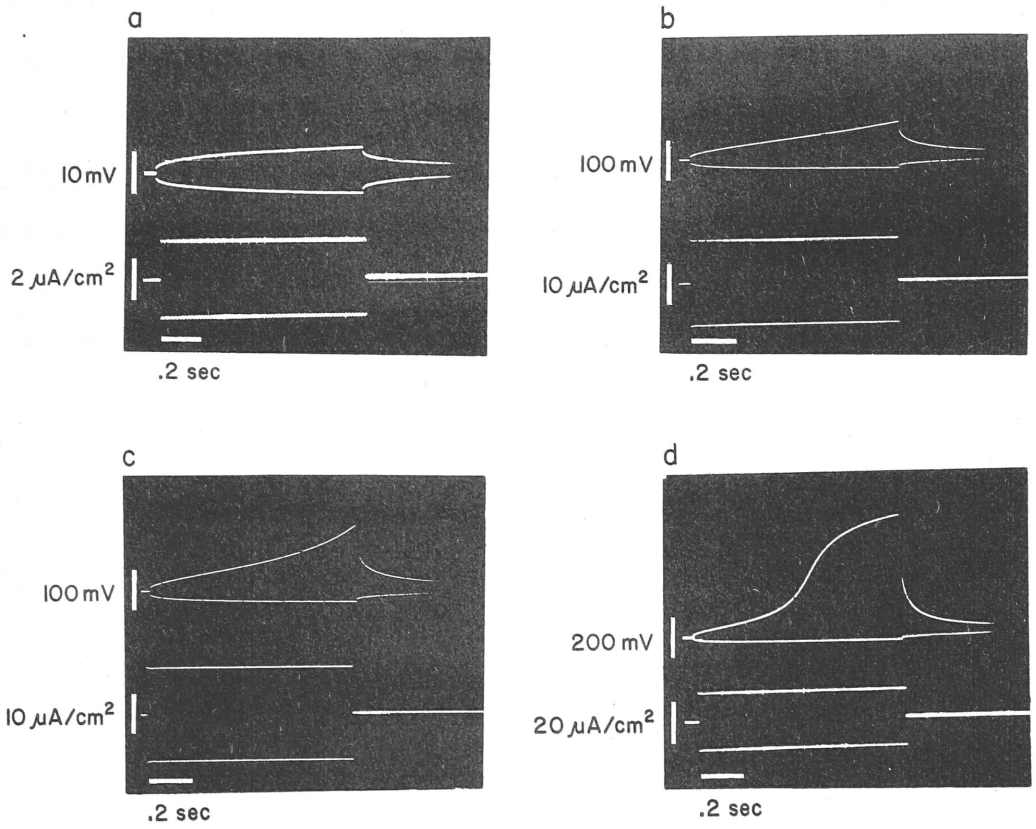

Figure 9. Records obtained on a bipolar AMFion membrane bathed on both sides by 0.1 N KCl. In each records the upper tracings indicate the voltage response; the lower tracings indicate the applied current step. The double tracings result from applying a positive current (upward deflection) and a negative current (downward deflection).

illustrated, therefore, the conservative aspect of the voltage produced by the current flow. Finally, for higher currents (Figures 9b, c, d) there is dramatic rectification. We see, therefore, that the characteristics predicted for a membrane having a sharp junction of positive and negative fixed-charge regions can be observed in the laboratory. With regard to the relevance of these phenomena to the plasma membrane, we may point out that if one accepts the Davson and Danielli model (i.e. that the essential membrane structure is that of a biomolecular layer of phospholipids), then, as a consequence of the choline and phosphate groups, there will be fixed-charge transition regions within the membrane.

PART II

In the preceding half of this paper, we have discussed some of the general properties of a diffusion regime of ions and have illustrated these with particular models. While the physical-chemical properties of ionic systems are inherently interesting in their own right, as physiologists we are particularly concerned with them because of the widely (although not universally) held belief that most of the electrical properties of cells, particularly the resting and action potentials, are manifestations of the permeability (or changes of permeability) of the plasma membrane to diffusible ions inside and outside of the cell. We wish to examine within the framework of the fundamental flux equations this presently held view, and then to discuss experiments on two particular biological systems, the frog skin and toad bladder, which we feel give insight into the basic problems confronting electrophysiologists.

[For the benefit of the reader not particularly familiar with the electrical phenomena associated with cells, let us here briefly describe them. (The remarks of this paragraph are of a very general kind and are by no means to be construed as applying to all cells.) Across the plasma membrane of the cell there exists an electrical potential difference, called the resting potential, of about 80 mV., with the inside of the cell negative with respect to the outside. Associated with the plasma membrane is a d.c. resistance of 1 kΩ per sq. cm. and a capacitance of 1 μF per sq. cm. In excitable cells, such as nerve and muscle, if current is passed so as to depolarize the cell (lower the magnitude of the existing potential) by about 12 mV., there is a regenerative change in the membrane potential wherein the potential rises rapidly to a peak value of 40 mV. (inside positive) and then falls back to the resting potential value. This transient change in potential, which occurs in a period of around a millisecond, is called the "action potential."]

A. Equivalent Circuits

The starting point of the modern ionic theory of the resting and action potential has been the equivalent circuit with the form shown in Figure 10 to represent the plasma membrane (Hodgkin and Huxley, 1952d). [We have omitted for simplicity the parallel capacitance element, which is not essential either for the theory or the analysis which we shall make of it.] From Kirchoff's Law we have for the individual ionic currents, I_j:

$$I_j = g_j (\Psi - E_j) \tag{39}$$

where E_j is called the "Nernst emf." and is by definition:

$$E_j = \pm \frac{RT}{F} \ln \frac{(c_j)_i}{(c_j)_0} \tag{40}$$

and of course the total current is given by:

$$I = \sum_j I_j \ .$$

[In (40) the + sign pertains to positive ions and the - sign to negative ions.] It should be understood that in the steady state, I_j is physically determinable, being the net amount of the j'th ion that crosses the membrane in unit time. [A double tracer experiment, if carried out with proper precaution, is a convenient way of getting I_j. In the squid axon, because of the rapidity of events, Hodgkin and Huxley (1952 a, b, c) had to identify "I_{Na}" and "I_K" by somewhat indirect means, but in principle these could have been identified directly.] Then it is clear that any ionic regime can be represented by the circuit in Figure 10, since g_j is an undetermined quantity, and hence can always be defined so as to satisfy (39). What then is the question? The question is what does g_j, which is formally the conductance of the j'th ion, mean physically in terms of a real ionic system, and just what aspects of the ionic system that it is meant to represent does the equivalent circuit of

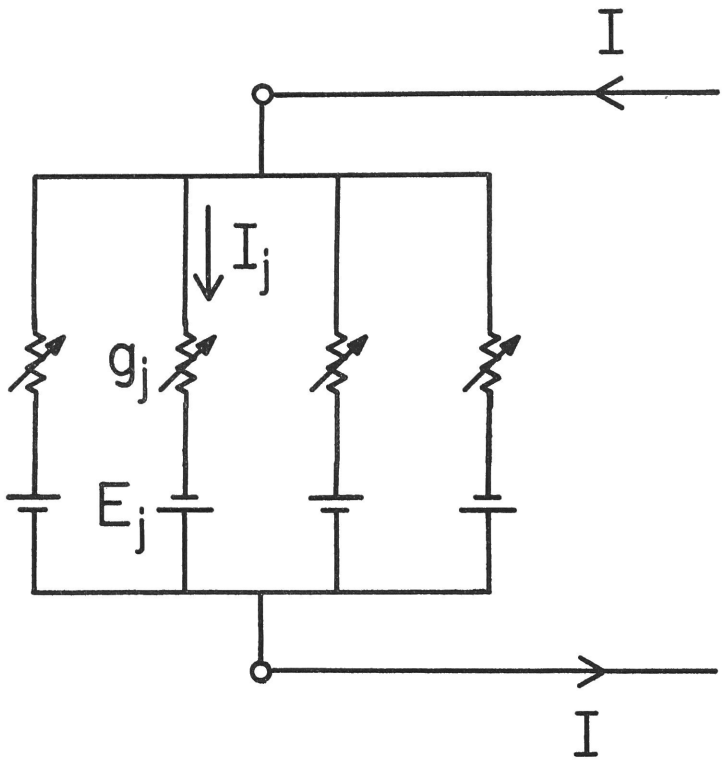

Figure 10. The type of equivalent circuit used among electrophysiologists to describe the electrical properties of the nerve membrane. Note that the emfs. are constant but that the conductances may be voltage-dependent. When used to represent a homogeneous membrane, this circuit is referred to as the "mixed equivalent circuit"; in that case g_j is the integral conductance of the j'th ion [equation (42)] and E_j is its "Nernst emf." [equation (40)].

Figure 10 actually represent? [These problems have been treated by Finkelstein and Mauro (1963) and the following analysis is largely a recapitulation of this paper.]

Let us begin by trying to answer these questions for the homogeneously uncharged membrane across which ions are diffusing subject to constrained boundary conditions [see section B of Part II]. We must start, as always, with the flux equations [equations (1a) and (1b)]. Rewriting (1a) we have:

$$\phi_j^+ = - u_j c_j^+ F \left(\frac{RT}{F} \frac{d \ln c_j^+}{dx} + \frac{d\psi}{dx} \right)$$

For the steady state, ϕ_j^+ is a constant throughout the membrane, and hence the above equation can be integrated to give:

$$\phi_j^+ \int_0^i \frac{dx}{F u_j c_j^+} = - \frac{RT}{F} \ln \frac{(c_j^+)_i}{(c_j^+)_0} - (- \Psi) \qquad (41)$$

where we have taken the potential ψ to be 0 in the outer solution and $- \Psi$ in the inner solution. Although it is only the algebraic sum of all ion flows across a membrane that is an electric current, we may formally consider the ion flux ϕ_j^+ as an electric current I_j^+ and define

$$E_j^+ \equiv \frac{RT}{F} \ln \frac{(c_j^+)_i}{(c_j^+)_0} \qquad (40)$$

as the "Nernst emf." of the j'th ion. Then, noting that

$$\int_0^i \frac{dx}{F u_j c_j^+}$$

is the integral resistance, R_j, of the j'th ion*, or

---

*The integral resistance is a consequence of integrating the specific resistivity

$$\frac{1}{F u_j c_j^+}$$

of the j'th ion as a function of x.

$$g_j \equiv \frac{1}{R_j} = \frac{1}{\int_0^i \frac{dx}{Fu_j c_j}} \tag{42}$$

the integral conductance of the ion, (41) can be rewritten in electrical language as:

$$I_j^+ = g_j (\Psi - E_j^+)$$

which is precisely equation (39) for cations. An identical expression can be derived from (1b) for anions, taking note of the fact that $I_k = - \phi_k^-$; i.e., a flux of negative ions in the positive direction constitutes a negative electric current. In this case E_k^- will be given by:

$$E_k^- \equiv - \frac{RT}{F} \ln \frac{(c_k^-)_i}{(c_k^-)_0} \tag{40}$$

We have thus answered our first question: Namely, that if we represent a homogeneously uncharged membrane in the steady state by the equivalent circuit of Figure 10, then g_j is the specific integral conductance of the j'th ion [equation (42)]. We note that the only restriction we have placed on our derivation is that the system has attained a steady state; thus, (39), with g_j defined by (42), will hold equally well for the free diffusion case, where no current is passed through the membrane, as for the case where a constant I (established by means of a pair of electrodes) is flowing across the membrane.

We now come to our second question: Namely, what properties of the homogeneous uncharged membrane does the circuit of Figure 10 describe? Clearly, from our above derivation, this circuit predicts the correct steady state $I - \Psi$ characteristic of the membrane, and further predicts the steady state flux, ϕ_j, of each ion. [It should be noted that we have, as yet, said nothing concerning the membrane resistance as compared to the equivalent circuit resistance; this problem will be considered a little later below.] It would seem at first glance, therefore, that there are no problems involved in using this circuit to describe a homogeneous, Planck diffusion regime of ions; in point of fact, however, there are several difficulties inherent in this

formalism.

In order to make clear the nature of these, let us turn back to equation (3c). [Note, that in our derivation of this equation, the only assumption we needed was that the divergence of I was zero. Thus, equation (3c) is valid for all states of the system, and, in contrast to (39), is not restricted to the steady state condition.] In our previous discussion of this equation [page 6], we observed that it expresses the fact that the total transmembrane potential, Ψ, is the sum of two terms which may be written as follows:

$$\Psi = IR_{int.} + \Psi_D \tag{43}$$

where, $R_{int.}$ is the total integral resistance of the membrane,

$$R_{int.} = \int_0^i \frac{dx}{F(U+V)} \tag{44}$$

and Ψ_D is the diffusion emf. arising from the ionic concentration gradients:

$$\Psi_D = \frac{RT}{F} \int_0^i \frac{d(U-V)}{(U+V)} \quad . \tag{45}$$

Equation (43) represents the type of equivalent circuit shown in Figure 11. This circuit describes all of the electrical properties of the membrane; i.e., it gives: the membrane potential, integral resistance, and diffusion emf. as a function of the current crossing the membrane; it gives no information, however, concerning ion fluxes. We thus see that there are two circuits which one may draw to represent a homogeneous membrane. For convenience, we shall henceforth refer to the equivalent circuit of Figure 11 as the "pure electrical equivalent circuit", since it pertains exclusively to all of the electrical properties of the membrane, while we shall call the equivalent circuit of Figure 10 the "mixed equivalent circuit", as it gives some information both about the electrical properties of the membrane and about the ion fluxes across the membrane. We are now in a position to discuss some of

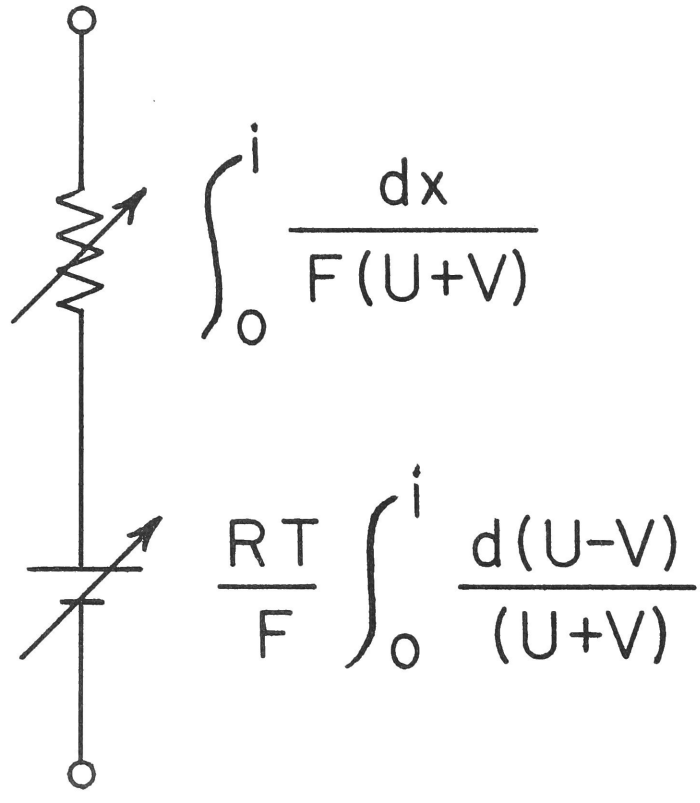

Figure 11. The "pure electrical equivalent circuit" for a homogeneous membrane. The resistance element is the total integral resistance of the membrane and the emf. is the diffusion emf. of the membrane. Note that both the resistive element and emf. may be voltage-dependent.

the paradoxes inherent in using the mixed equivalent circuit for describing a homogeneous membrane.

Single Salt Case. [Figures 12a and 12b]. Turning first to the single salt case, we have, upon substituting (9) into (42), the individual ionic conductances:

$$g^{+} = \frac{1}{\int_0^i \frac{dx}{Fuc}} = \frac{\frac{Fu}{\delta}(c_i - c_0)}{\ln \frac{c_i}{c_0}} \tag{46a}$$

$$g^{-} = \frac{1}{\int_0^i \frac{dx}{Fvc}} = \frac{\frac{Fv}{\delta}(c_i - c_0)}{\ln \frac{c_i}{c_0}} \tag{46b}$$

We note that these conductances are not functions of I; this is a consequence of the fact that the ionic profiles will not be disturbed by the passage of current. If we calculate from the mixed equivalent circuit the free diffusion potential ($\Psi_{I=0}$), by noting that

$$I^{+} = g^{+}(\Psi - E^{+}) = \frac{\frac{Fu}{\delta}(c_i - c_0)}{\ln \frac{c_i}{c_0}} \left(\Psi - \frac{RT}{F} \ln \frac{c_i}{c_0}\right)$$

$$I^{-} = g^{-}(\Psi - E^{-}) = \frac{\frac{Fv}{\delta}(c_i - c_0)}{\ln \frac{c_i}{c_0}} \left(\Psi + \frac{RT}{F} \ln \frac{c_i}{c_0}\right)$$

we have, since $I = 0$,

$$I^{-} + I^{+} \equiv I = 0 = \frac{\frac{F}{\delta}(c_i - c_0)}{\ln \frac{c_i}{c_0}} \left(u\Psi_{I=0} + v\Psi_{I=0} - \frac{RT}{F} u \ln \frac{c_i}{c_0} + \frac{RT}{F} v \ln \frac{c_i}{c_0} \right)$$

and finally,

$$\Psi_{I=0} = \frac{RT}{F} \frac{u - v}{u + v} \ln \frac{c_i}{c_0} \tag{4 }$$

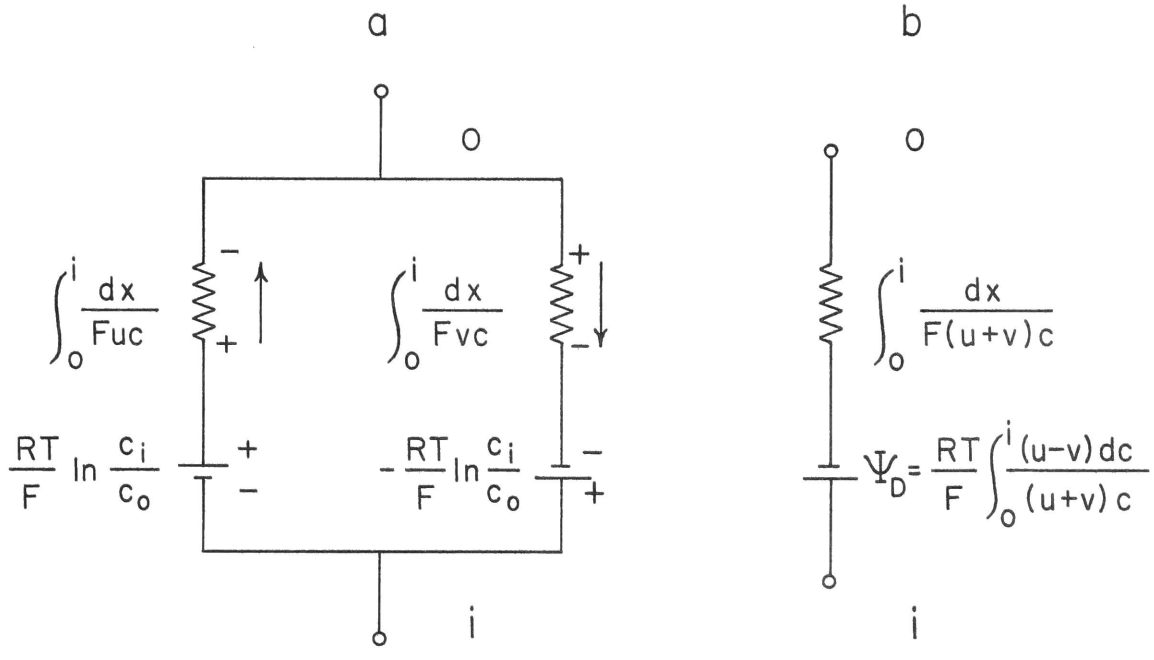

Figure 12. (a) Mixed equivalent circuit for the single salt case. Note that the resistances are voltage-independent.

(b) Pure electrical equivalent circuit for the single salt case. Note that both the resistance and emf. are voltage-independent.

which is the familiar result obtained by solving the flux equations by the conventional methods.

What is now of interest is an examination of the manner in which the free diffusion potential, $\Psi_{I=0}$, arises in the mixed equivalent circuit as compared to its actual physical origin in the diffusion regime. In the latter instance, due to the difference in mobilities of the cation and anion, an electric field is set up within the membrane which acts on all ions, so that, at any point in the membrane, the ions diffuse as a consequence of the gradient of their chemical potential

$$(RT\frac{1}{c}\frac{dc}{dx})$$

and of the gradient of electrical potential

$$(F\frac{d\psi}{dx}) .$$

Furthermore, at any point in the membrane the cation and anion fluxes are equal, and no electric current flows in the membrane. In the mixed equivalent circuit, on the other hand, the ionic diffusion fluxes are interpreted as real currents, the cation being driven by $(\Psi - E^{+})$ acting across its integral resistance and the anion being driven by $(\Psi - E^{-})$ acting across its integral resistance. Within the circuit, local currents flow with IR drops appearing across the two resistance elements. Certainly, the physical situation depicted by the mixed equivalent circuit is quite different from the one actually existing. To really emphasize this point, consider the case where the cation and anion mobilities are identical (KCl is a good approximation for this situation). In this case, both the direct consideration of the diffusion regime in the membrane and the mixed equivalent circuit give $\Psi_{I=0} = 0$. But in the former case, no state of electrification exists anywhere in the system, while in the latter, despite the fact that $\Psi_{I=0} = 0$, there exist internally local currents, two IR drops, and two emf.'s, the emf.'s being equal to the IR drops [see Figure 13]. Thus, despite the fact that

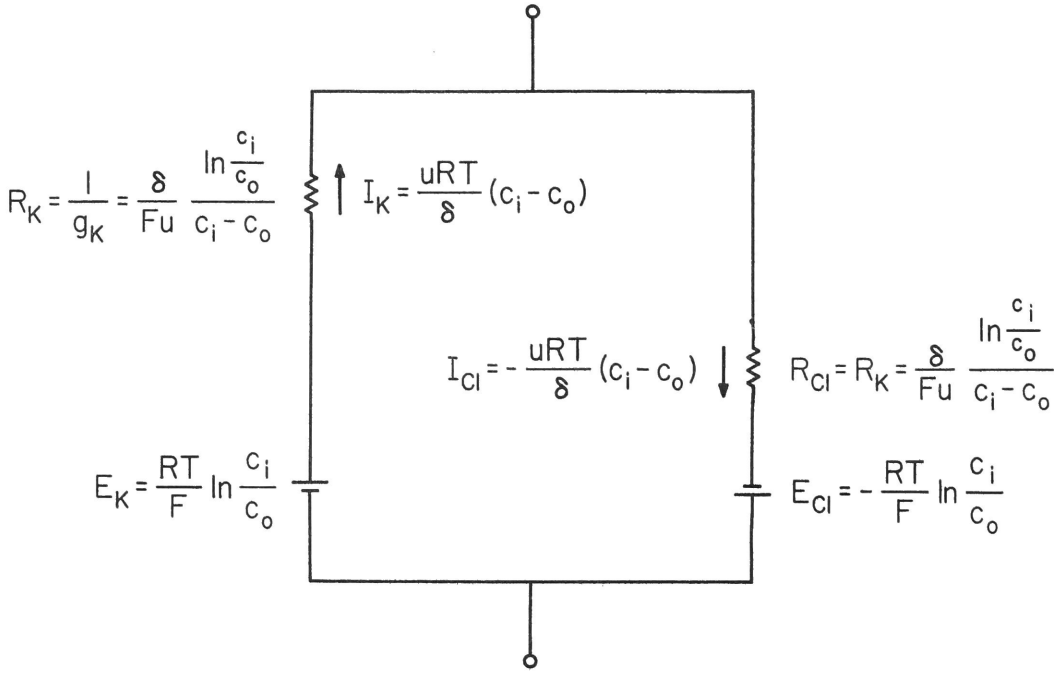

Figure 13. The mixed equivalent circuit, with no external current flowing through the system, for the single salt case where the cation and anion have equal mobilities (approximated by KCl). Note that although the potential across the membrane is 0, there are internal currents, emfs, and IR drops.

the mixed equivalent circuit predicts the correct fluxes and membrane potential, the physical conditions implicit in the circuit are far from those existing within the membrane. Of course, being an "equivalent" circuit, it makes no pretense of necessarily saying anything about the physics of the membrane process; it is just this point which must be continually kept in mind. Note, however, that if instead of a homogeneous membrane we were dealing with a "mosaic" membrane consisting of some regions exclusively permeable to cations and other regions exclusively permeable to anions, the same equivalent circuit (Figure 12a) would, in fact, accurately represent the actual physical processes by which ions crossed the membrane, namely, through local currents, as indicated, flowing in each branch pertaining to the region for a given ionic species.

Turning to the pure electrical equivalent circuit for the single salt case (Figure 12b), we find for free diffusion no fictitious currents as arose above in the mixed circuit; indeed, in this "pure" representation, the current in the circuit is zero for free diffusion, i.e. $I = 0$. We simply have a diffusion emf.

$$\Psi_D \equiv \frac{RT}{F}\int_0^i \frac{d(U-V)}{(U+V)} = \frac{RT}{F}\int_0^i \frac{(u-v)dc}{(u+v)c} = \frac{RT}{F}\,\frac{u-v}{u+v}\,\ln\frac{c_i}{c_0} \tag{12}$$

in series with the total membrane integral resistance [which may be evaluated with the help of (9)]:

$$R_{int.} \equiv \int_0^\delta \frac{dx}{F(U+V)} = \frac{1}{F(u+v)}\int_0^\delta \frac{dx}{c} = \frac{1}{F(u+v)}\int_0^\delta \frac{dx}{\frac{(c_i-c_0)}{\delta}x + c_0}$$

$$R_{int.} = \frac{\delta}{F(u+v)(c_i-c_0)}\ln\frac{c_i}{c_0} \tag{11}$$

For the case in which the anion and the cation mobilities are identical, we see from (12) that $\Psi_D = 0$ and the equivalent circuit is just an ohmic resistance with no internal state of electrification, which is precisely the

situation within the diffusion regime of ions. Clearly, then, as far as the electrical state of the membrane is concerned, the pure electrical equivalent circuit is a more accurate representation than the mixed circuit.

Equal Total Concentration of Ions on the Two Sides of the Membrane.

Our remarks for the single salt case concerning the erroneous physical impression that the mixed equivalent circuit conveys apply equally well to any more general cases and need not be repeated. There is, however, a further problem that arises as a consequence of the voltage dependence of the ionic profiles. This has to do with the method of depicting the non-linearity in the voltage-current relationship [see page 11]. In the mixed equivalent circuit, the membrane potential is artificially represented only by IR terms and constant emfs. Thus, the non-linear properties of the membrane are depicted as due solely to the non-linear nature of the g's (Figure 10). But consideration of equation (43) [along with (44) and (45)] reveals that the non-linearity of the membrane potential actually will be due, in general, not only to the non-linearity of the membrane conductance ($1/R_{int.}$), but also to a change in the basic emf. (Ψ_D) of the membrane.* [See Appendix II for the specific dependence of these quantities on Ψ.] In other words, both the resistance and the emf., Ψ_D, depend on the concentration profiles, and in the relaxation of the system from one state to another they will be time-variant. The pure electrical equivalent circuit faithfully depicts this (Figure 11), but the mixed equivalent circuit fictitiously lumps both of these phenomena together as time-variant conductances. On the

*In those cases where the total concentration of ions is the same on the two sides of the membrane, but there is only one anion (or cation) involved in the system, it can be shown that Ψ_D, the diffusion emf., will be invariant to current flow; the special case in this class occurs where three species are present, one ion being common, and is usually referred to as the "bi-ionic" case.

other hand, if we were dealing with a mosaic membrane consisting of regions of complete selectivity for individual ions, the mixed equivalent circuit not only would give the correct values for Ψ and the ionic fluxes (which it, of course, does for the homogeneous membrane), but would also convey an accurate physical description of the system. [In fact, the branches in the mixed circuit would refer to local homogeneous regions.] Thus, any non-linear properties of the mosaic membrane would be entirely due to non-linear behavior of the separate conductances.*

Let us elaborate a little more on the contrast between the "mixed" and "pure" equivalent circuits. Summing (39) over all species we have:

$$\Psi = I \frac{1}{\sum_j g_j} + \frac{\sum_j g_j E_j}{\sum_j g_j} \qquad \text{(mixed equivalent circuit)} \qquad (48)$$

where E_j and g_j are given by equations (40) and (42), respectively. On the other hand,

$$\Psi = IR_{int.} + \Psi_D \qquad \text{(pure electrical equivalent circuit)} \qquad (43)$$

where $R_{int.}$ and Ψ_D are defined by equations (44) and (45), respectively. We have shown that both (48) and (43) give the same steady state characteristic for the membrane. Now, at first glance, there seems to be a term for term correspondence between the two equations; this, however, is not correct! By inspection, we see that in general

$$\frac{1}{\sum_j \int_0^i \frac{dx}{F u c_j}} \neq \frac{1}{F} \int_0^i \frac{dx}{\sum_j u_j c_j^+ + \sum_k v_k c_k^-}$$

---

*In a mosaic membrane consisting of permselective regions for individual ions, the voltage dependence of the individual conductances would not be due to the shifting of ionic profiles, since in each region there is only one permeant ion. The conductances, however, could change as the result of an increase or decrease in the number of regions, or through various other effects. Independent of the mechanism, the branched circuit would be appropriate.

that is, the resistance of the mixed equivalent circuit is not the integral resistance of the homogeneous membrane it is representing.* Similarly, the diffusion emf. (Ψ_D) of the membrane is not given by the second term in (48).* As a matter of fact, the two terms in (48) in general have no relevance to any directly measurable electrical property of the homogeneous membrane; they are two formal quantities that add together to give for any value of current, I, the correct membrane potential, Ψ.

To demonstrate this last point with a particularly dramatic example, let us consider the situation shown in Figure 14a, in which we have equal total concentrations of $K^+ + NH_4^+$ on the two sides of the membrane and only one anion, A^-. If we assume that A^- has a very small mobility and therefore a negligible integral conductance [and of course, its Nernst emf. is zero], the mixed equivalent circuit will be given by Figure 14b. At I = 0, there is, of course, zero membrane potential ($u_{NH_4} \approx u_K$), and the mixed equivanlent circuit correctly predicts this in that $g_{NH_4} = g_K$ and $E_{NH_4} = - E_K$. Now, if high enough current is passed from left to right, in the limit K^+ will have a steady state concentration of 0.1 N in the membrane and NH_4^+ a concentration of 0.01 N. If the circuit is now suddenly opened, what is the situation? Across the actual membrane there obviously is still zero potential difference ($\Psi_D = 0$), since NH_4^+ and K^+ are (postulated) electrically identical. But, from the mixed equivalent circuit, we would predict, by the second term in (48) that:

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*An exceptional case is the single salt case where the two will be equal. This is easily shown mathematically but is even more obviously seen physically, since this is the one case where ionic profiles do not shift with current. Thus,

$$\frac{1}{\sum_j g_j}$$

is constant for all times and is clearly the integral resistance of the membrane. Similarly,

$$\frac{\sum_j g_j E_j}{\sum_j g_j}$$

is, in this case, the diffusion emf. of the membrane.

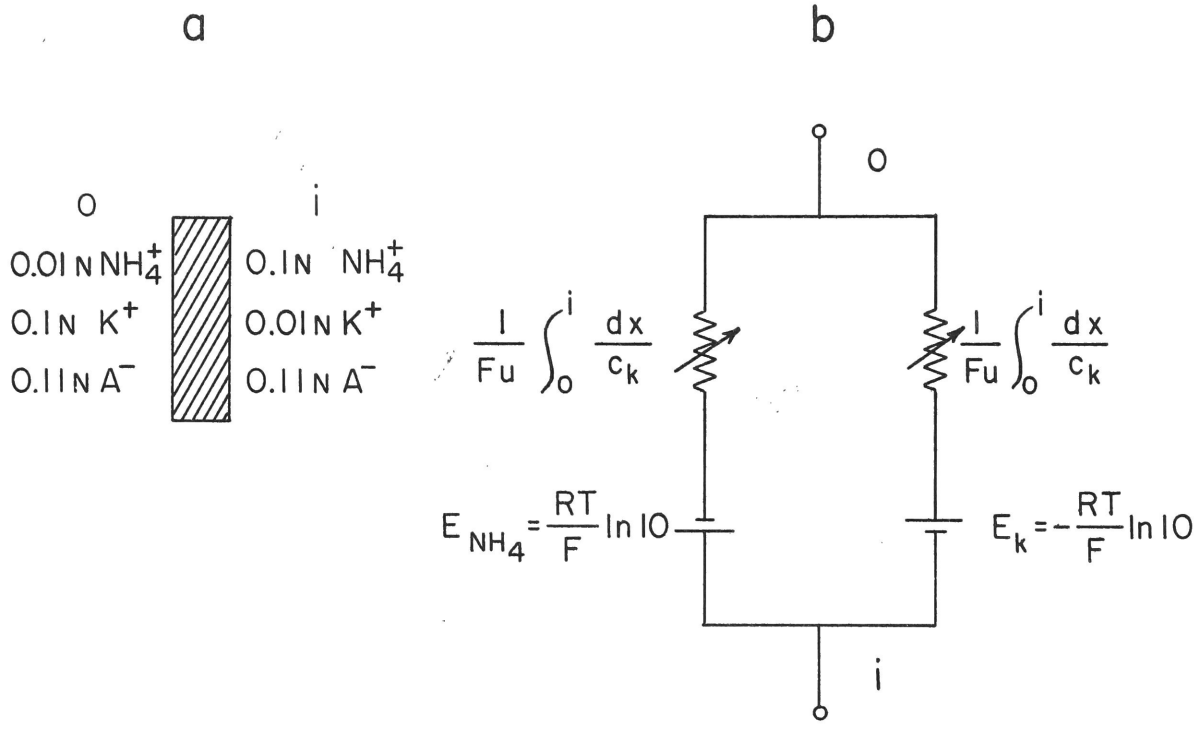


Figure 14. (a) Homogeneous membrane separating the two solutions indicated. The mobility of the anion A^- is assumed to be approximately 0.

(b) The mixed equivalent circuit for the system depicted in Figure 14a.

$$\Psi = \frac{0.1\,E_K - 0.01\,E_K}{0.1 + 0.01} = \frac{0.09}{0.11}\,\frac{RT}{F}\,\ln\frac{0.1}{0.01} \approx 50 \text{ mV} \quad \text{(room temperature)}$$

Thus, clearly the second term in (48) is not Ψ_D. [From a mathematical standpoint the reason that (48) does not give the correct result is that this equation is only valid for the steady state condition, and when we open the circuit a steady state no longer exists, the ions requiring time to relax back to the steady state $I = 0$ condition.]

Let us now try to relate what we have done so far, with the present day ionic theory of the resting and action potential in a nerve axon. As we mentioned before, the starting point of the theory is the equivalent circuit of the form shown in Figure 10. Essentially, there are two branches in the circuit pertaining to the axon- the sodium branch and the potassium branch. (see Figure 33a). The sodium emf. and potassium emf. have opposite polarities as a consequence of the fact that the potassium concentration inside the axon is higher than the concentration in the surrounding fluid, and conversely for the sodium ion. The negative resting potential results (according to the ionic theory) from the potassium conductance being much higher than the sodium conductance. These conductances, however, are voltage-dependent, and when sufficient current is passed through the membrane to depolarize the membrane to the threshold potential, there occurs a regenerative increase in the sodium conductance until it actually becomes higher than the potassium conductance. This sodium conductance change will profoundly change the membrane potential, as can be seen by applying Kirchoff's Law to the equivalent circuit. A little later in time the sodium conductance begins to fall toward its resting level and still later there occurs an increase in potassium conductance. Both of these changes tend to bring the membrane potential back to the resting value. Thus, the action potential results from relative changes in the value of the sodium and potassium conductances.

Granting that the action potential is indeed due to changes in the membrane permeability to sodium and potassium, the question is whether

the equivalent circuit of Figure 10, which we are using to calculate the effect of these permeability changes on the membrane potential, is describing a homogeneous or a mosaic membrane; that is, is the axonal membrane homogeneous in the sense that sodium and potassium go through the same regions or does it consist of separate sodium and potassium permeable patches? Our previous analysis has shown that we can use the same equivalent circuit, i.e., Figure 10, in either case. If the membrane is homogeneous, however, then, despite the fact that the equivalent circuit attributes the action potential to conductance changes, it is in reality due to a change in the basic emf. of the system, since the action potential is occurring with no current flowing in the membrane. On the other hand, if the membrane is a mosaic, then indeed the action potential is exclusively the result of the conductance changes which modulate the local currents flowing in the membrane.

The question as to whether the action potential is basically a resistance change or an emf. change is by no means trivial, if one is interested in getting at the physics underlying the phenomenon. It therefore is of some interest to consider two biological systems, the frog skin and toad bladder, in which there occurs an "action potential" that is, as we shall show, fairly unambiguously attributable to a regenerative resistance change which can be completely decoupled from any intrinsic emf. in these systems. The remainder of this paper will be devoted to a description and discussion of these two systems. [A brief report on the "action potential" in frog skin and toad bladder has appeared previously (Finkelstein, 1961).]

B. The Frog Skin and Toad Bladder

1. Material and methods. All of the experiments to be described in this section were performed *in vitro* on the abdominal skin of the frog *Rana pipiens* (occasionally *Rana temporaria*) and on half bladders of the toad *Bufo marinus*; they were conducted over a two-year period on both male and female animals during all seasons of the year. In most experiments, the preparation was mounted in the two-compartment lucite chamber shown

in Figure 8, and current-voltage measurements were performed in the same manner as described for the artificial membranes (page 30). In control experiments, it was found that the results were unaffected by stirring in the two compartments; consequently, in most cases stirring was dispensed with for the sake of convenience. In those experiments where nitrogen and air were bubbled through the solutions bathing the preparation, the chamber shown in Figure 15 was used.

So that the reader may be oriented with respect to the histological structure of the skin and bladder, we show in Figures 16a and 16b diagramatic cross sections of them. While both of these are complex multicellular organs, it is generally recognized that the layer of primary electrical and physiological importance is the epithelial layer (Ussing, 1948; Leaf et al., 1958). In the bladder, this is a single sheet of epithelial cells (Figure 16b), while in the skin it is a more complex structure containing a cornified layer, and even some multicellular glands (Figure 16a); the germinatival layer of the epidermis, however, which is one or two cell layers thick, is regarded as the physiological important one. In the discussion, we shall return to the consideration of the site of the electrical responses which we observe, but we wished to alert the reader at the outset that, despite the complexity of these two organs, the region of interest is a single (or at most double) layered sheet of epithelial cells. In both organs, these cells perform very similar functions; namely, they are involved in regulating the movement of salt and water into and out of the animal.

2. Results. Within an hour after mounting either preparation in the chamber, a steady state d.c. potential of from 20 to 100 mV. was obtained across the system with Ringer's solution as modified by Gray* on

*71.75 mM NaCl; 5.00 mM KCl; 2.50 mM $CaCl_2$; 1.60 mM $MgCl_2$; 20.0 mM $NaHCO_3$; 1.03 mM Na_2HPO_4; 0.12 mM NaH_2PO_4; 0.26 mM glucose. The solution is saturated with a 97% O_2, 3% CO_2 gas mixture.

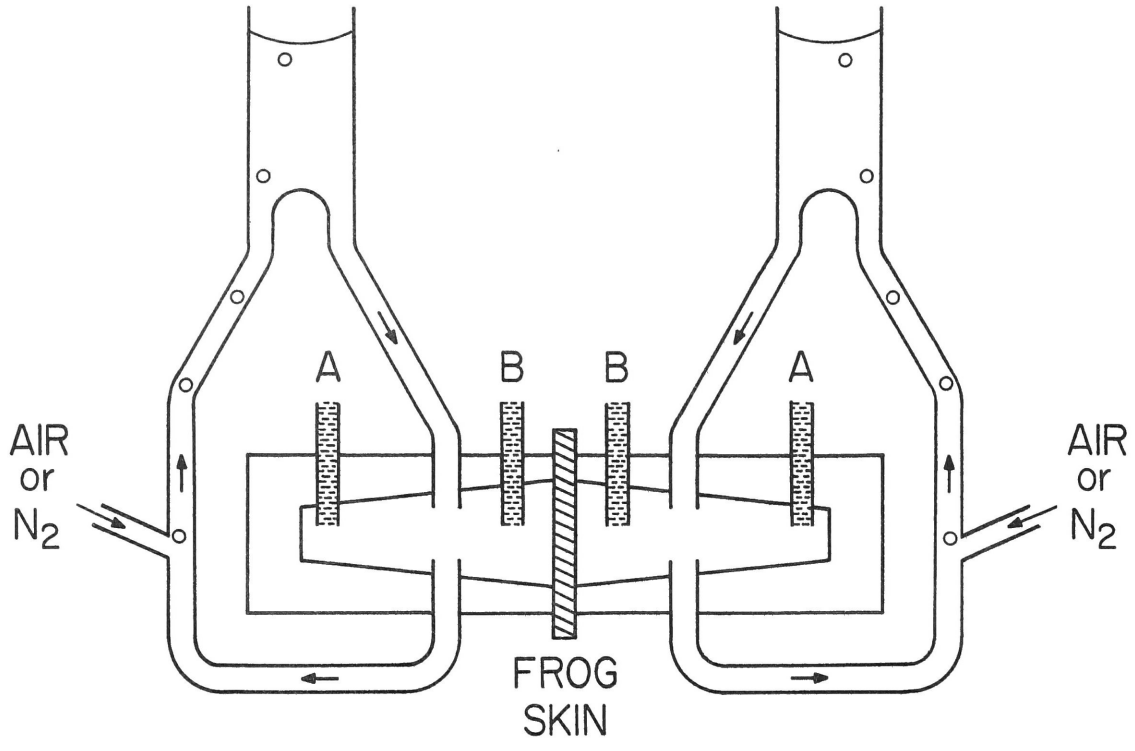
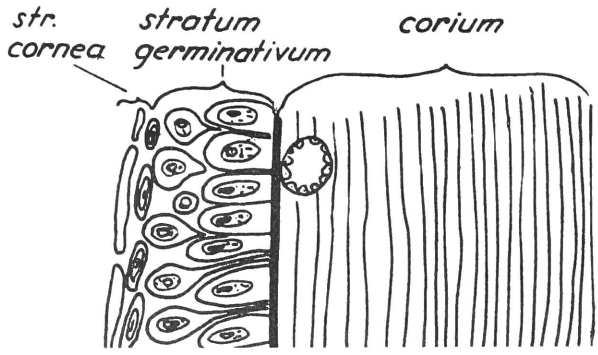


Figure 15. Schematic diagram of chamber used in anoxia experiments. A are the agar-KCl bridges used for passing current; B are the agar-KCl bridges going to the recording electrodes.

a



b

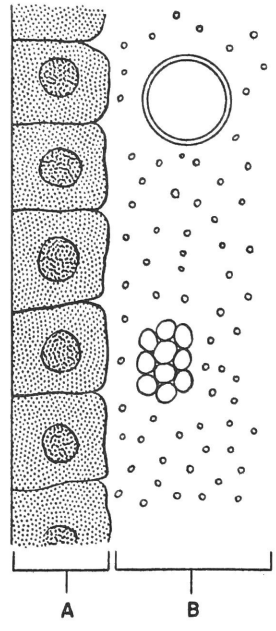


Figure 16. (a) A schematic drawing of a section through the frog skin showing the different layers. (From Koefoed-Johnsen and Ussing, 1958).

(b) Schematic representation of a cross-section of the toad bladder. Section marked A is the single layer of bladder epithelial cells at the mucosal (luminal) border of the membrane. B is a layer of loose connective tissue made up of collagen fibers, smooth muscle bundles, and capillaries. The serosal limiting membrane has been omitted from the figure. (From Frazier, 1962).

both sides. In the skin, the serosal (inside) surface is positive with respect to the anatomical outside; in the bladder the serosal surface is positive with respect to the mucosal (lumenal) side.

a. The basic responses: Figure 17 shows a typical set of responses of the skin to square waves of current of 35 msec. duration. For small values of inward (depolarizing) and outward (hyperpolarizing) current* the voltage responses are symmetrical (Figure 17a), and the skin is phenomenologically equivalent to a network consisting of a resistance and capacitance in parallel (parallel RC network). The resistances observed range from 0.2 - 1.0 $k\Omega/cm^2$, and the capacitances range from 2 - 5 $\mu F/cm^2$. As the magnitude of the current pulse is increased, the potential changes begin to show non-linearity and rectification, with the larger response occurring for outward current (Figure 17b). At still higher current densities, the system is very non-linear, and for an outward current pulse, overshoot (a graded response) occurs (Figure 17c). As the magnitude of the current pulse is continually increased, a value is eventually reached at which the skin is near threshold; this value occurs around 300 mV. At this point, if the magnitude of the current pulse is increased slightly, the skin responds with an "action potential" for inward current pulses, while continuing to give an overshoot for outward currents (Figure 17d). [In some skins, instead of an overshoot response for outward currents, there occurs an "action potential" with a sharp threshold and a form essentially the mirror image of the type shown in Figure 17d; this is illustrated in Figure 18.] In Figure 19, the response is shown for current of longer duration. We note that there is not repetitive firing, although a second, graded oscillation does occur.

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*By an inward current we shall mean a current produced with the cathode in the solution bathing the inner surface of the skin (serosal surface of the bladder) and the anode in the solution bathing the outer surface (mucosal surface of the bladder); the reverse is the case for outward current.

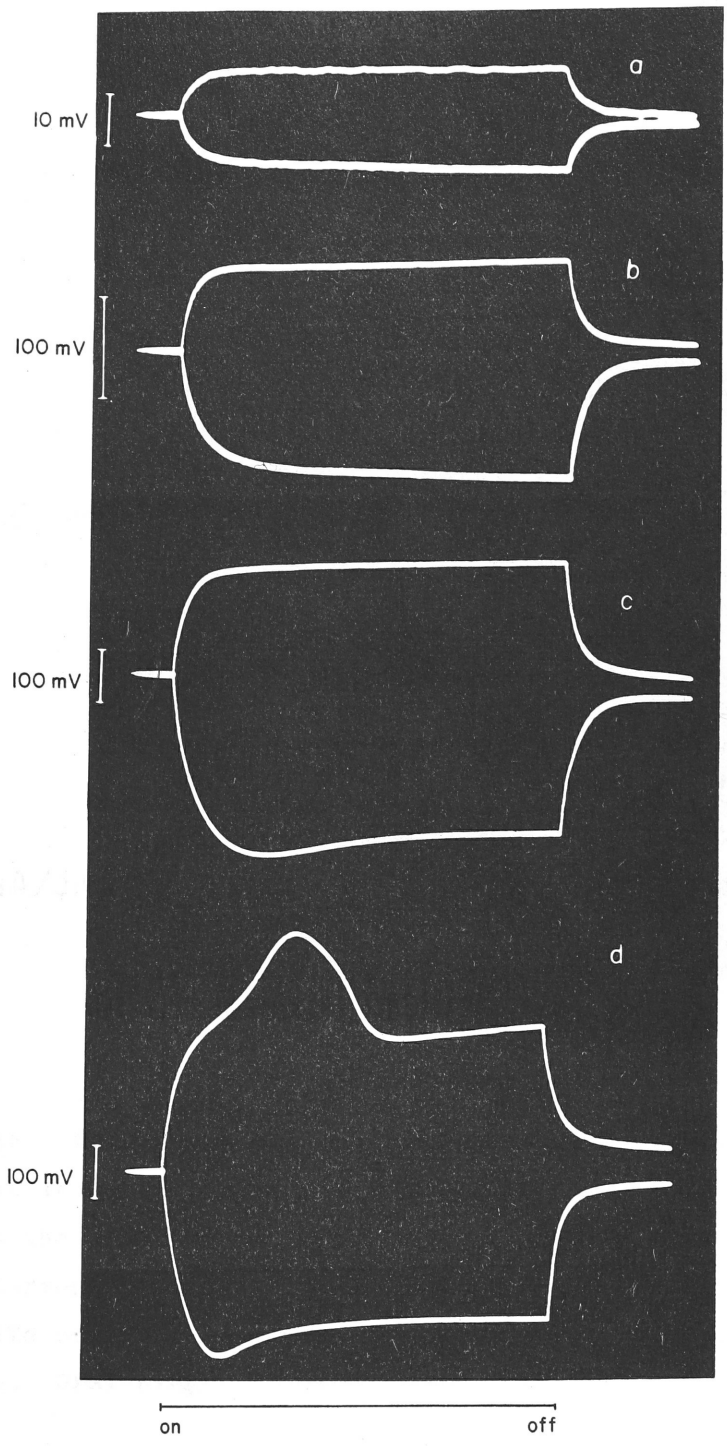

Figure 17. The change in potential across isolated frog skin produced by 35 msec. square waves of current. Upward response is the result of inward current; downward response is the result of outward current. Currents are 20 μ amp./cm^2, 210 μ amp./cm^2, 500 μ amp./cm^2, and 550 μ amp./cm^2 for a, b, c, and d, respectively. Resting potential is 30 mV., inside positive. Gray-Ringer solution on both sides.

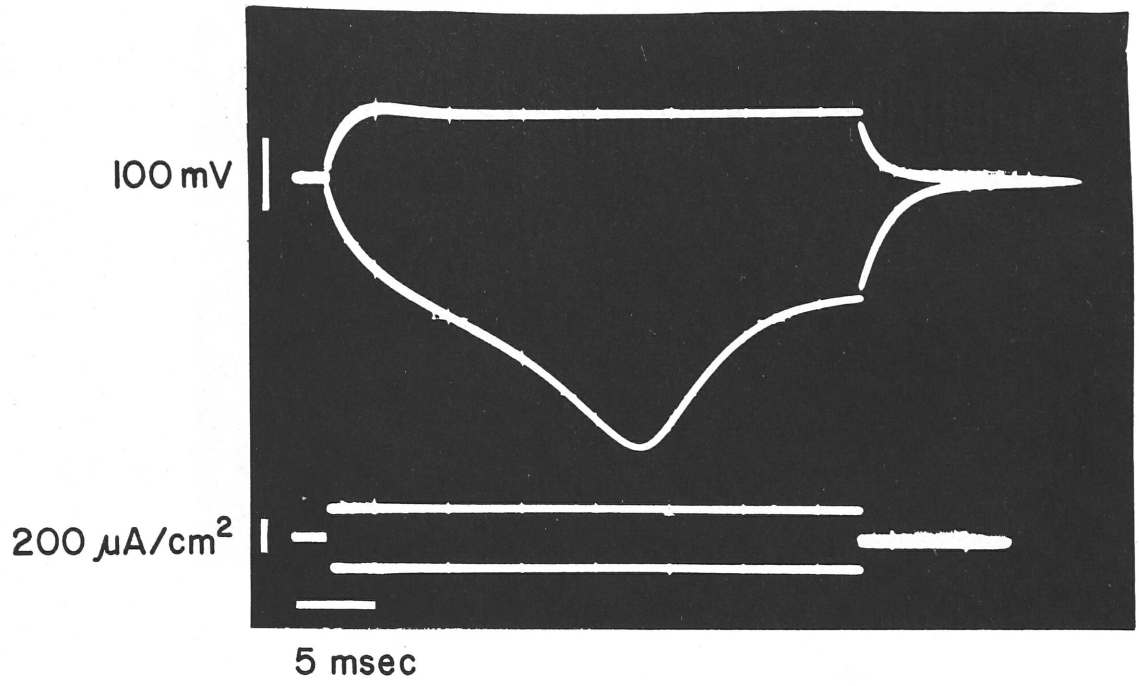

Figure 18. Record illustrating an action potential for outward current across isolated frog skin. The upward response is the result of inward current; the downward response is the result of outward current. (At higher current densities, an action potential of the form shown in Figure 17d occurs for inward current.) Resting potential is 50 mV; inside positive. Gray-Ringer on both sides.

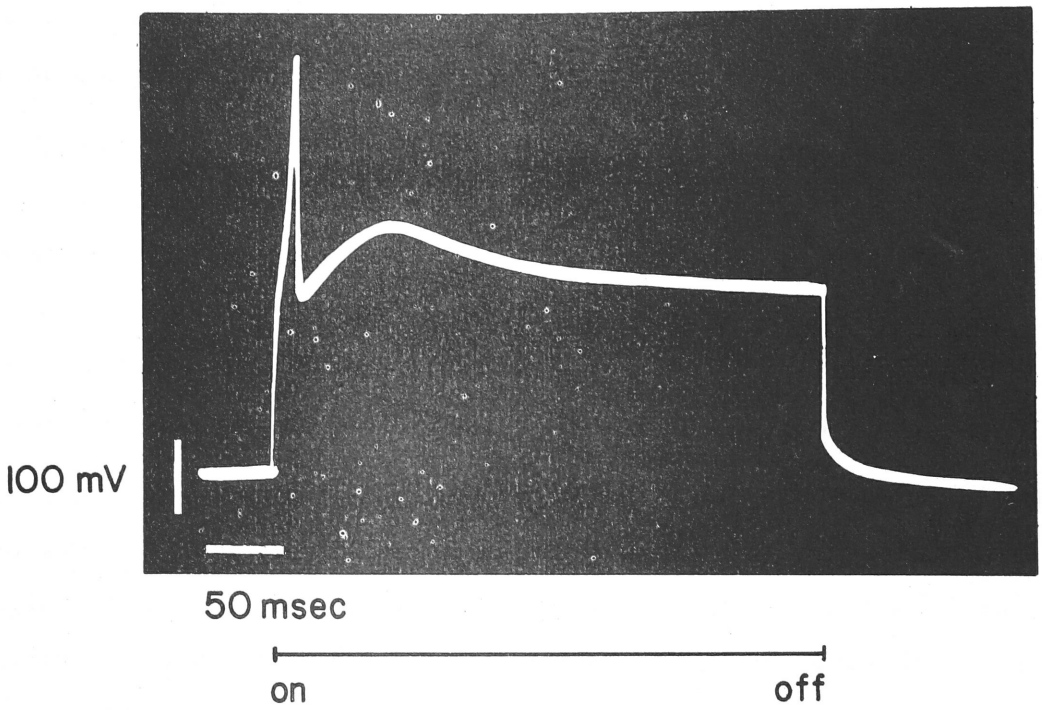

Figure 19. The action potential across isolated frog skin in response to a prolonged (350 msec.) inward current of intensity 700 μ amp./cm^2. (Note the difference in time scale as compared to Figure 17.) Resting potential is 32 mV; inside positive. Gray-Ringer solution on both sides.

Following an action potential, the skin becomes refractory for several seconds(5 - 20 seconds), not exhibiting an active response to the same stimulus that previously elicited it (Figure 20). If during this period the skin is stimulated at the rate of 10/sec. or faster, it will continue to remain refractory and show only an RC-type of response; stimulation at the rate of 1/sec. or less, on the other hand, has no appreciable effect on the duration of the refractory period.

In Figure 21 is seen a striking example of the recovery of excitability following a full response. In this experiment, a small short (2.5 msec.) pulse is superimposed on a 40 msec., just sub-threshold pulse. The interval between successive records is three seconds. Curves a, b, and c show a characteristic sub-threshold "local response", curve d is the full action potential. A pulse three seconds after this would give curve a again, etc.

Turning to the toad bladder, we find qualitatively the same behavior as described above for the frog skin. The resistances of the bladders are generally higher than those of the skins, ranging from 0.75 - 2.5 $k\Omega/cm^2$, but the capacitances are within the same range of values as in the skin. In Figure 22 we see the action potential occurring for inward current, and a small overshoot response for outward current. [Unlike the skin, we have never observed an action potential with a sharp threshold for outward current.] Note that the action potential of the bladder is of longer duration than that for the skin; and in particular, the falling phase is considerably extended in time. As in the skin, the refractory period is of several seconds duration, but it is generally not so long as in that system, being only about 5 seconds.

Very often the bladder preparation failed to exhibit a response with a sharp threshold for inward current, displaying instead a graded overshoot behavior. The full all or none response could frequently be potentiated, however, by first repetitively stimulating for several seconds with threshold currents at a frequency of 10/sec. or faster. This phenomenon is illustrated in Figure 23. It was observed that during the repetitive current stimulation

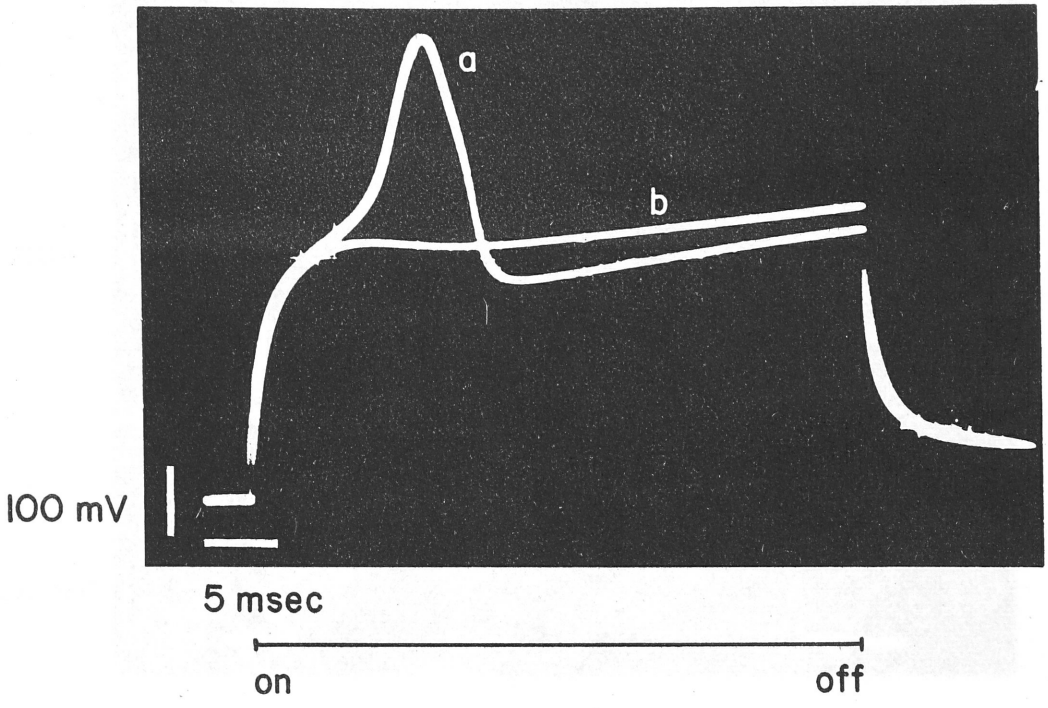

Figure 20. Record illustrating the refractory period following an action potential across isolated frog skin. Both tracings are in response to an inward current of 800 $\mu a/cm^2$; tracing b was obtained 5 seconds after a. Resting potential is 35 mV, inside positive. Gray-Ringer solution on both sides.

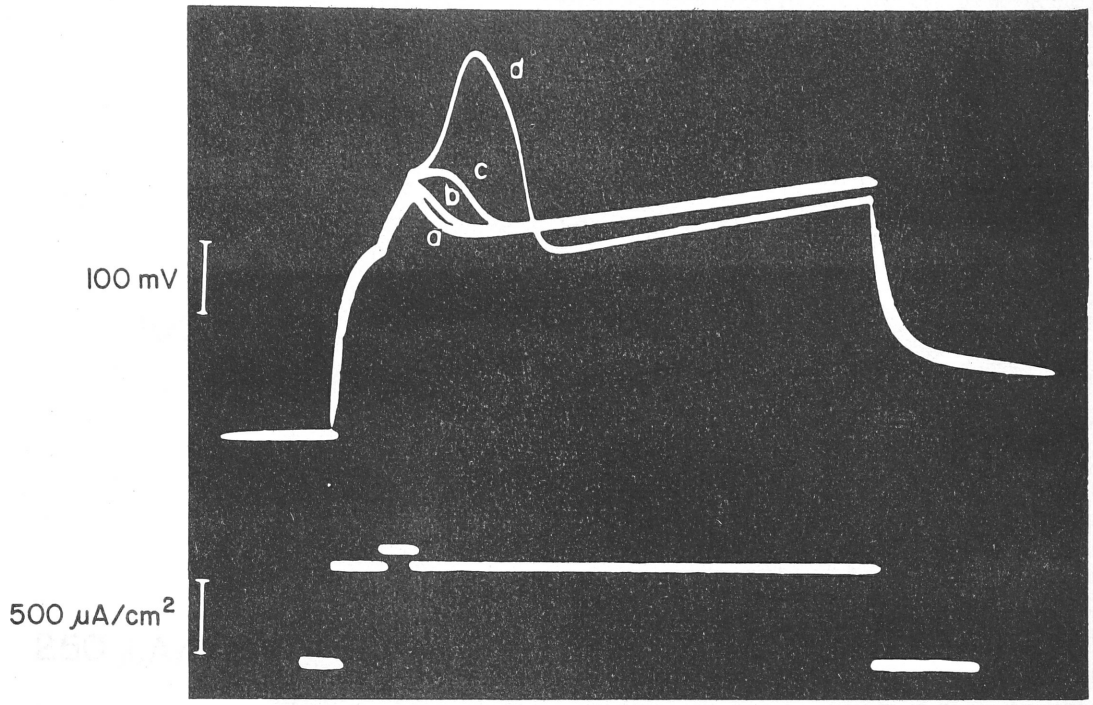

Figure 21. The change in potential across isolated frog skin produced by a 40 msec. just sub-threshold inward square wave of current with a superimposed 2.5 msec. small inward square wave. Successive responses a, b, c, and d are 3 sec. apart. Resting potential is 60 mV, inside positive. Gray-Ringer solution on both sides.

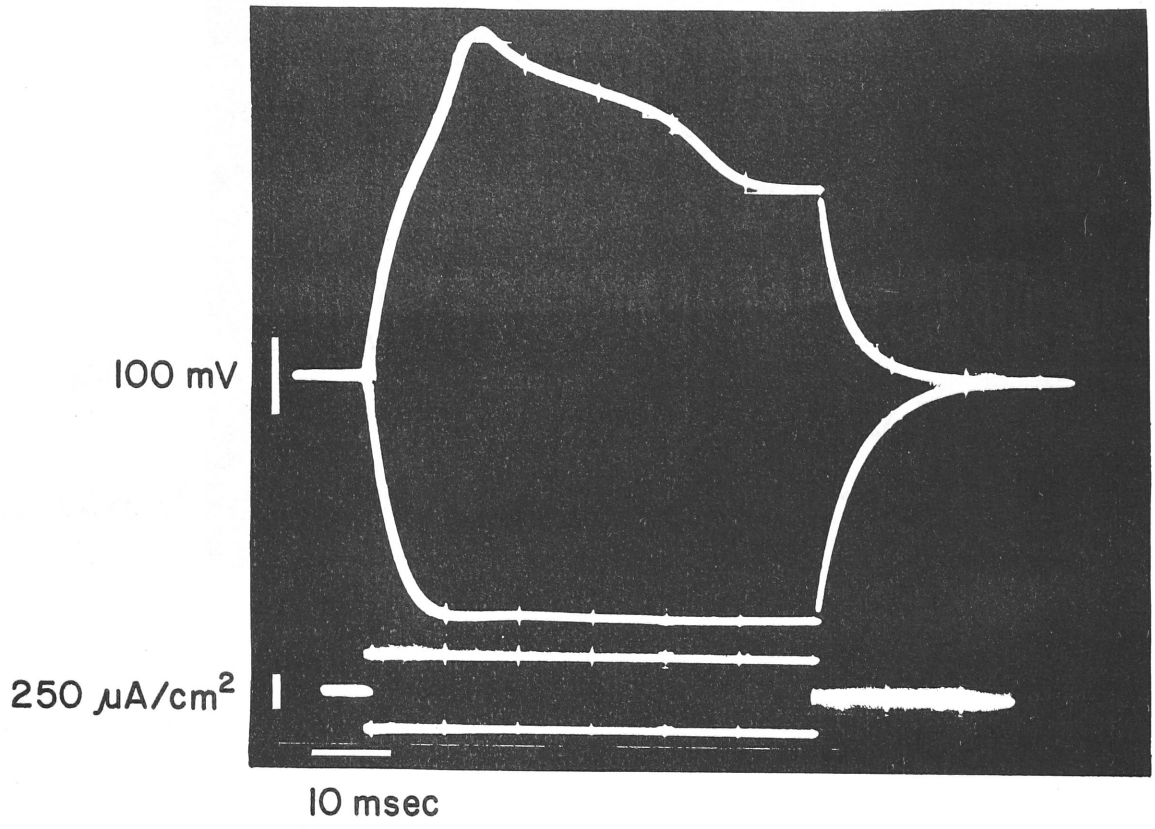

Figure 22. The action potential across isolated toad bladder in response to a 60 msec. square wave of super-threshold current. The upward response (action potential) is the result of inward current; the downward response is the result of outward current. Resting potential is 80 mV, serosal side positive. Gray-Ringer solution on both sides.

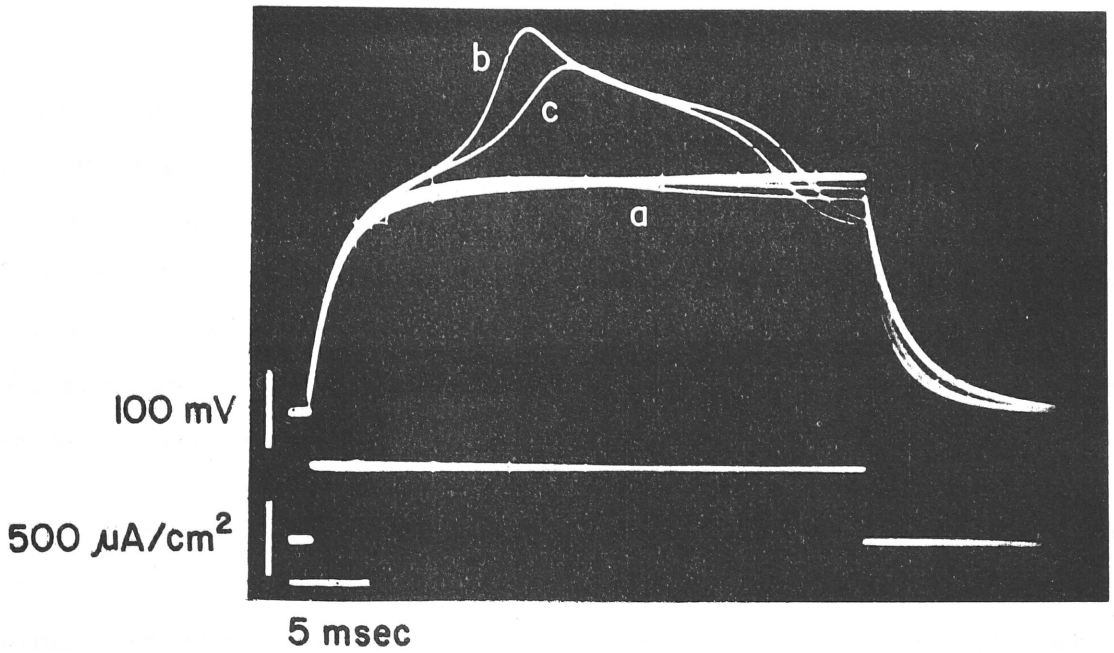

Figure 23. The production of action potentials across isolated toad bladder following repetitive stimulation. Initially, this preparation was unexcitable. Following repetitive stimulation at the rate of 10/sec. for 20 seconds, with a sufficiently large inward current (in this case 500 μa/cm^2), the preparation became excitable. In this record the intensity of the repetitive stimulating current and of the current producing the action potential was the same. The a tracings were obtained during the repetitive stimulation; tracings b and c were obtained 4 and 5 seconds, respectively, after the repetitive stimulation. Note the short refractory period, as evidenced by the fact that tracing c was obtained one second after b. This is a distinguishing feature of a bladder that has been repetitively stimulated. Resting potential is 90 mV., serosal side positive. Gray-Ringer solution on both sides.

the magnitude of the resulting trans-bladder voltage decreased; we shall comment later on this point.

To summarize, then, the general description of the phenomenon with which we are dealing in frog skin and toad bladder, we may state that when the potential across the preparation, bathed on both sides by Gray-Ringer solution, has reached an appropriate value (about 300 mV inside negative in the skin and about 200 mV serosal side negative in the bladder), there occurs at a sharp threshold an "action potential" of from 100 to 300 mV magnitude. Following this response, the preparation is refractory for several seconds duration.

b. The resistive nature of the responses: If at any time during the occurrence of the action potential (in either the skin or bladder) the stimulating current is interrupted, the response fails to proceed, and the potential across the preparation rapidly decays to its resting level (Figure 24). [It is interesting that a refractory period does not develop if the current is interrupted at some time during the early or middle part of the rising phase of the spike. Refractoriness begins to appear during the end of the rising phase, and if the current is removed at any time after the peak in the potential has been reached, a full length refractory period follows. It appears, therefore, that the events responsible for refractoriness are those associated with the production of the falling phase of the response.] Thus, the action potential occurs only in the face of a steady state current passing across the preparation. This immediately suggests that the response we are observing is due to the fact that the preparation is behaving as a time variant resistance; that is, the voltage changes we are seeing in the face of a steady state current are a manifestation of the modulation of the current by a varying resistance element.

In order to determine whether this was indeed the case, a bridge measurement was performed (Figure 25). The frequencies used to operate the bridge were 1000-2000 cps., and the maximum voltage introduced across

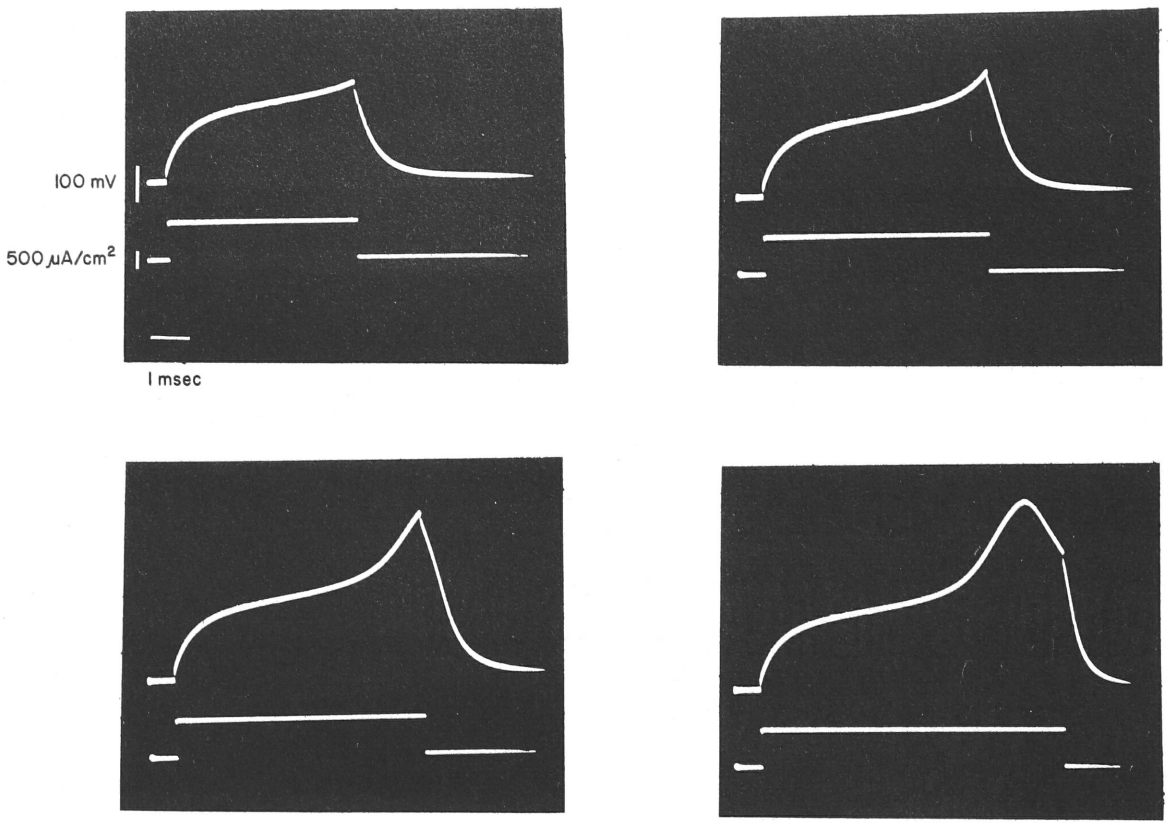

Figure 24. Records demonstrating that removal of the current during any phase of the action potential causes an interruption of the response. (These records were obtained on frog skin; similar results are obtained for the toad bladder.) Gray-Ringer solution on both sides.

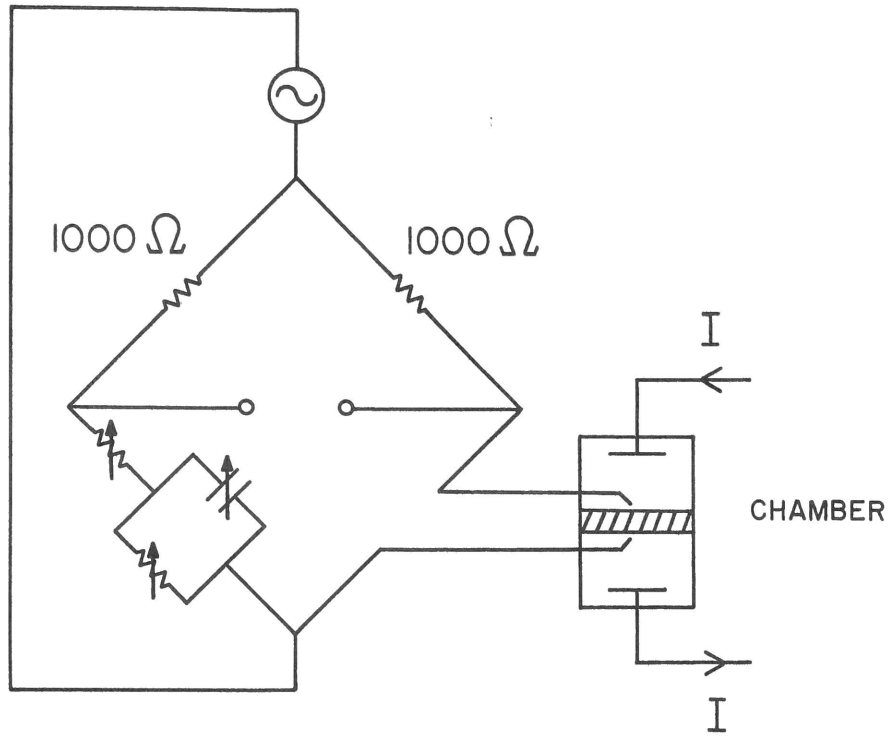

Figure 25. Bridge used for measurements of resistances across frog skin and toad bladder.

the preparation by the measuring current was less than 10 mV. The measurements were performed as follows: the bridge was first balanced for a just sub-threshold stimulating current. Then, the simulating current was increased slightly so as to produce an action potential. During the action potential the bridge became unbalanced, and this could be seen superimposed on the response. In order to determine the direction and magnitude of the unblanace, the bridge was balanced at various times during the response. This could always be accomplished by changing the parallel resistance element in the reference arm with little change in the capacitance and series resistance element in that arm. By such a procedure it was found that during the early and middle part of the rising phase of the response, the resistance increased, and that following this it began to fall, actually reaching a value below that which existed during the sub-threshold period. These results are completely in accord with and confirm the view that the voltage response we are observing is due to a time variant resistance. [Notice that the fact that at the end of the spike the potential falls to a value below that of the sub-threshold potential (see Figures 20 and 23) is the result of the resistance falling below the sub-threshold value. We now see also that the decrease in the transbladder voltage response during repetitive stimulation (Figure 23) is the result of a fall in the transbladder resistance; once this low resistance state has been reached, the previous graded response is converted to an all or none response. That is, once in the low resistance state, the bladder can be electrically stimulated to pass in an all or none manner to the high resistance state.]

c. The current-voltage characteristic: In Figure 26 is shown a plot of the peak voltage response of the frog skin as a function of current. [A similar curve is obtained for the toad bladder.] Notice that the height of the action potential barely increases with increasing current stimulation. (The effect of increased current is to shorten the latency of the active response.) This means that although the spike is produced by an increased total chord resistance of the preparation, the slope resistance at the peak is

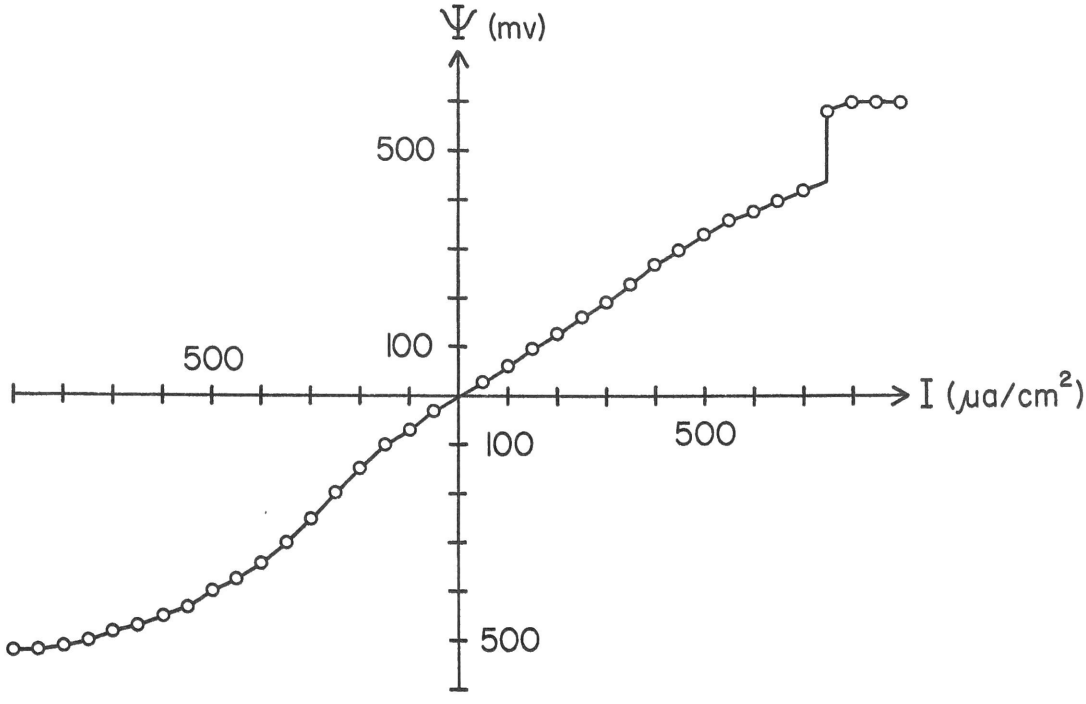

Figure 26. The peak voltage response across frog skin as a function of current. The duration of the current pulse is 180 msec. The abrupt rise in voltage at 750 $\mu a/cm^2$ inward current indicates the occurrence of the action potential. Resting potential is 45 mV, inside positive. (The curve has been displaced upward 45 mV. on the Ψ -axis.), Gray-Ringer solution on both sides.

markedly decreased. In fact, we see in Figure 27 that following the action potential, the skin remains in a state of small slope resistance, which in a sense is another manifestation of the refractory period. We further observe in Figure 26 that also for large outward currents the slope resistance of the skin decreases. Since in this region of the graph the peak of the graded overshoot response is being plotted, we may infer (along with the fact that in some skins an all-or-none action potential occurs for outward currents) that the graded response is not basically a different phenomenon from the all-or-none response.

d. The effect of changing the ionic compositions of the solutions:* As a general result we may state that alteration of the ionic composition of the solution bathing the inner surface of the skin produces negligible effects on the action potential. These alterations include substitution of all of the sodium by potassium or rubidium, varying the calcium concentration from 0 to 30 mM, and substitution on an equivalence basis of all of the chloride by sulphate. [In the calcium experiments, the irreversible loss of excitability sometimes occured upon prolonged (30 minutes or longer) exposure to calcium-free solutions or to high calcium concentrations.] On the other hand, the active response is markedly affected by similar changes in the ionic composition of the solution bathing the outer surface, and it is these results which we shall now discuss.

We may first mention that a "normal" all-or-none action potential occurs when virtually all of the chloride is replaced by sulphate on either

*In this section the experiments to be discussed will be confined to the frog skin. The reason for this is that the all-or-none action potential is much less frequently obtained in the bladder than in the skin, where it is almost always present. Furthermore, alterations of the ionic composition in the bladder preparation frequently produce irreversible changes, and in this section we are particularly interested in reversible effects.

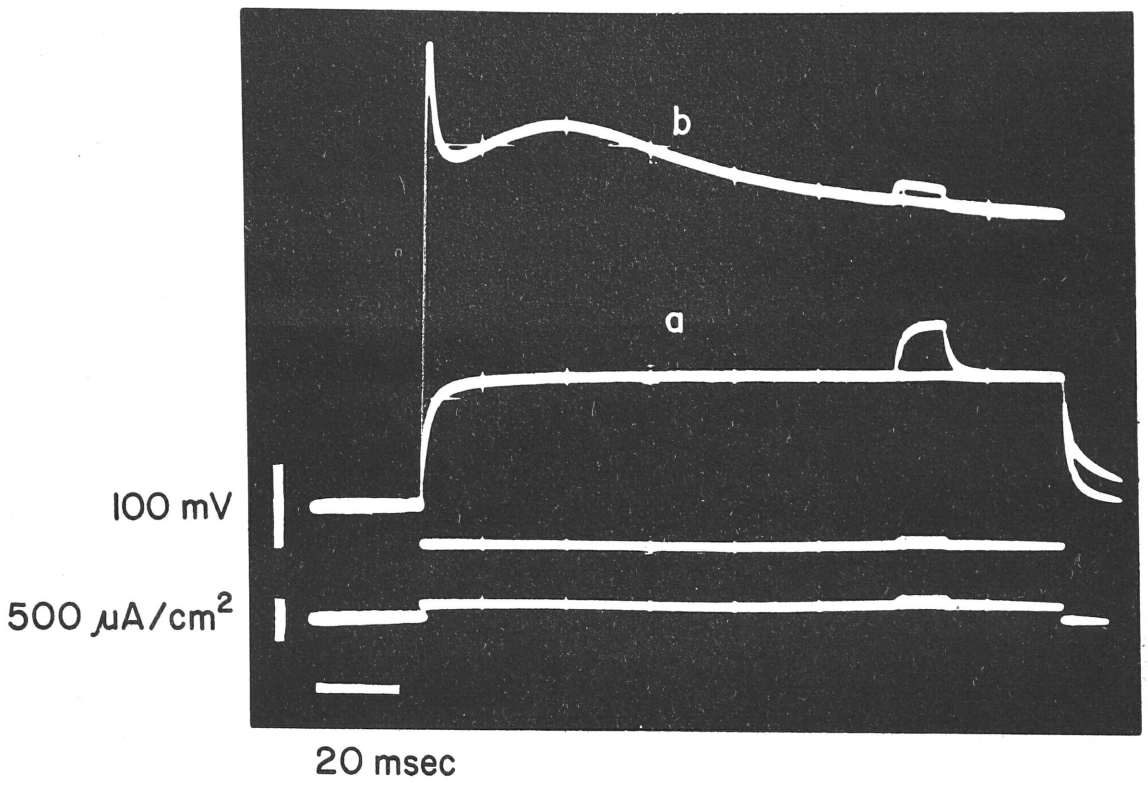

Figure 27. Record illustrating the small slope resistance of the frog skin following an action potential. Superimposed upon a 160 msec. inward square wave of current is a small 10 msec. inward square wave. Note that the same 10 msec. pulse produces a much larger voltage response (tracing a) when added on a sub-threshold current than when added on a current pulse that has given rise to an action potential (tracing b). Resting potential is 60 mV, inside positive. Gray-Ringer solution on both sides.

one side or both sides of the skin. In Figure 28 we see such a response with sulphate Ringer on both sides. In several experiments we replaced the sulphate on an equivalent basis by metasulphate without observing any marked change in the response.

If the Na^+ concentration is reduced on the outside to 20 mM either with or without replacement by K^+, there is little change in the active response. Below this level, however, the response begins to lose its all-or-none character and becomes graded. In Figure 29, we see the result of replacing all of the Na^+ by K^+ in the outer solution. We observe that the small signal resistance of the preparation markedly increases when K^+ replaces Na^+ (compare Figures 29a and 29c), and that for large inward current, the all-or-none action potential has changed to a graded overshoot response (Figures 29b and 29d). For comparison we see in Figure 29f that replacement of Na^+ by K^+ in the inner solution does not produce a change in the all-or-none character of the response. We wish to point out that the alteration in the response produced by substitution of K^+ for Na^+ (as seen in Figures 29c and 29d) on the outside occurs within the time it takes to change the solutions (not more than 10 seconds). Furthermore, the effect is completely reversible. Even after the skin has been exposed for over two hours to the sodium-free solution, replacement of the K^+ by Na^+ leads "immediately" to a return of the system to its previous state; that is, the resistance drops to its normal value and the all-or-none response reappears.

The character of the response is dramatically affected by the concentration of Ca^{++} in the outside solution. If the Ca^{++} concentration is reduced to 0, the threshold of the action potential rises considerably, and in about half of the experiments the all-or-none character is lost, the response becoming graded. This is particularly true if the potassium concentration is also brought to 0 along with the Ca^{++}. A typical set of records is seen in Figure 30. The full magnitude of the effect produced by removal of Ca^{++} from the outer solution requires from 5 to 15 minutes to develop, although an observable effect is seen within a few seconds after removal of Ca^{++}. The effect of the

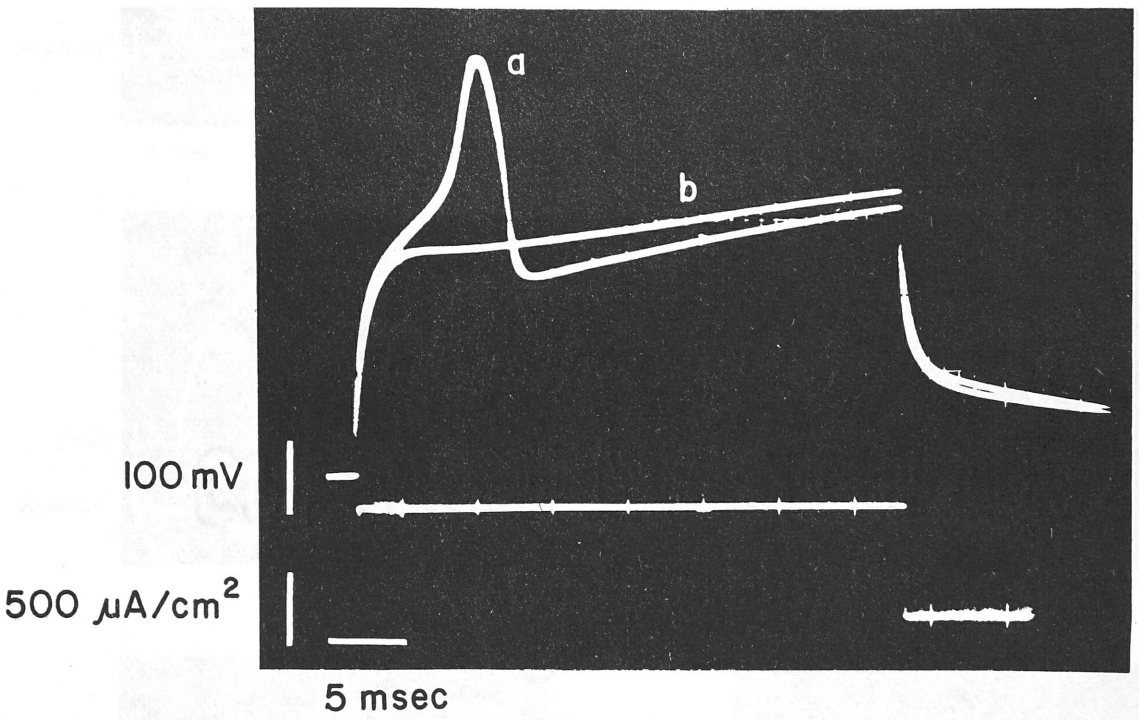

Figure 28. Demonstration of action potential across isolated frog skin when bathed on both sides by sulphate Ringer (100 mN Na_2SO_4; 5 mN K_2SO_4; 2.5 mM $CaCl_2$). Tracing b was obtained 3 seconds after a. Resting potential is 68 mV, inside positive

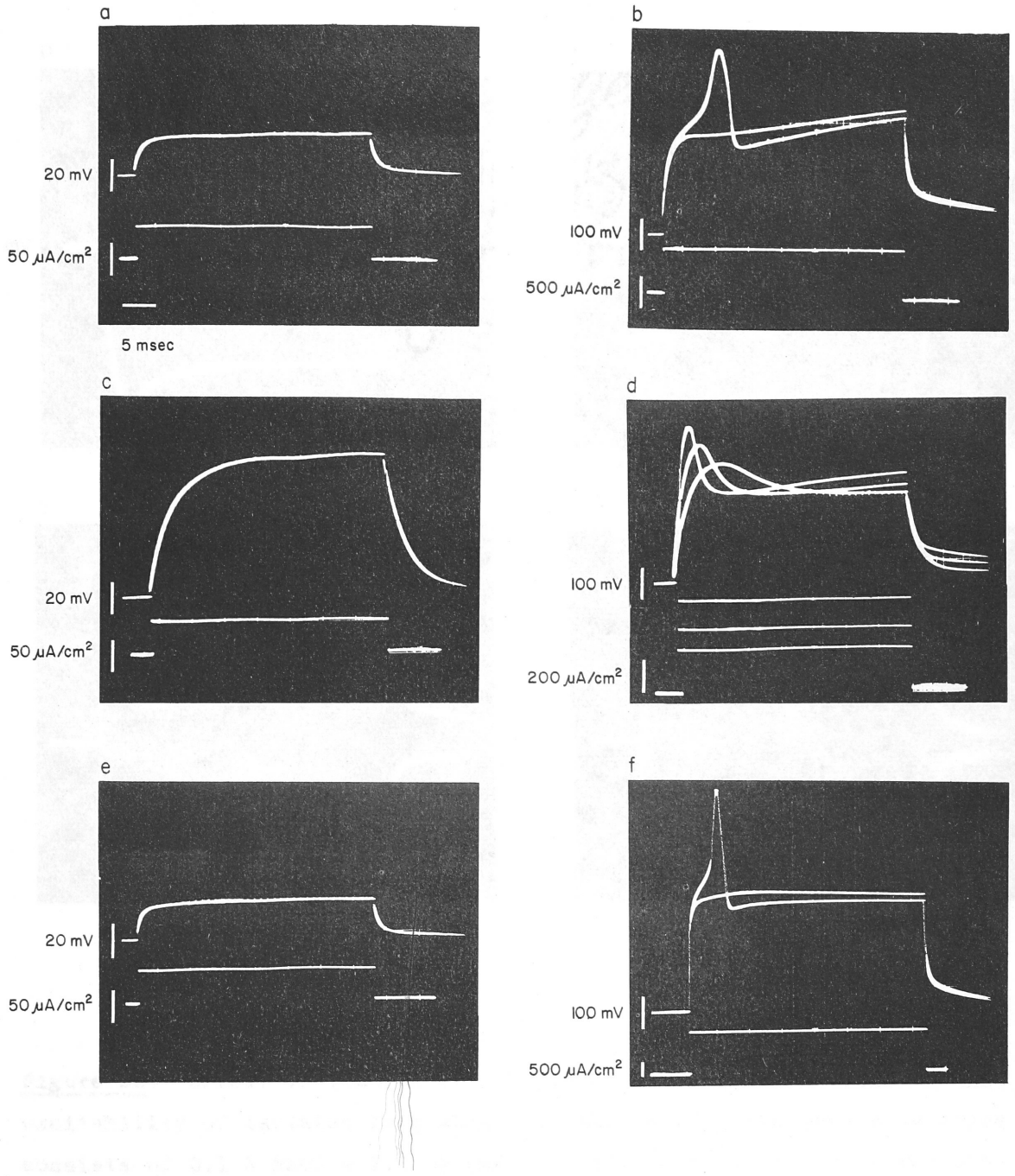

Figure 29. Results of substituting K^+ for Na^+ on the inside and outside of isolated frog skin. In records a and b the solution on both sides is sulphate Ringer (100 mN Na_2SO_4; 5 mN K_2SO_4; 2.5 mM $CaCl_2$); in records c and d the inside solution is still sulphate Ringer, but the outside solution has been changed to 100 mN K_2SO_4 + 2.5 mM $CaCl_2$; in records e and f, the outside solution has been changed to sulphate Ringer and the inside solution to 100 mN K_2SO_4 + 2.5 mM $CaCl_2$.

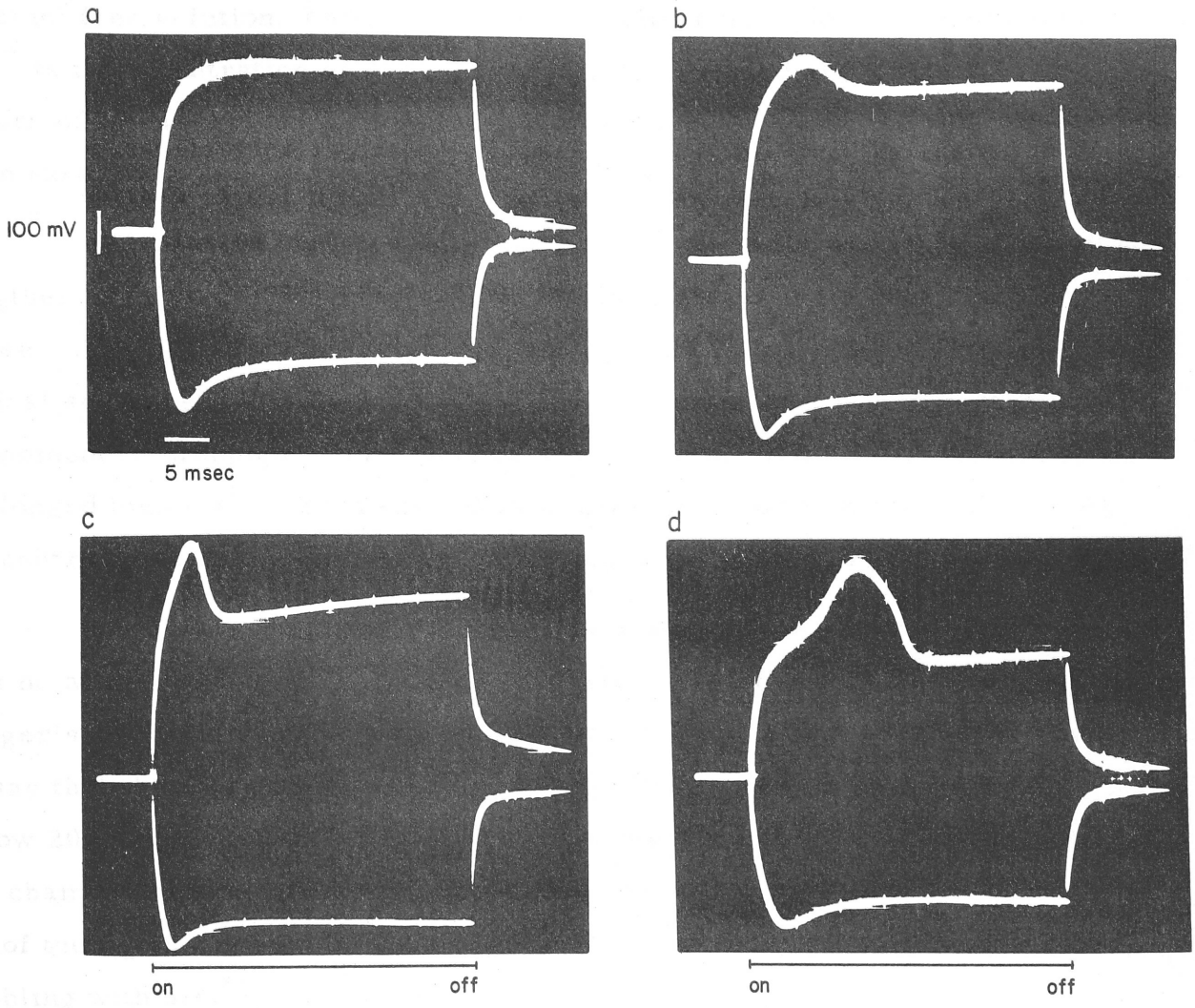

Figure 30. Effect of removal of Ca^{++} from the outside solution on the excitability of isolated frog skin. In all records the inside solution consists of 0.1 N NaCl + 2.5 mM $CaCl_2$. In records a, b, and c the outside solution contains only 0.1 N NaCl. Record d was taken 10 seconds after the outside solution had been changed to 0.1 N NaCl + 2.5 mM $CaCl_2$. The currents are 700 μ amp/cm^2, 800 μ amp/cm^2, 900 μ amp/cm^2, and 550 μ amp/cm^2 for a, b, c, and d, respectively. (In all records the upward tracing is the response to inward current and the downward tracing is the response to outward current.)

calcium free solution, however, is completely reversible; replacement of Ca^{++} to a concentration of 2.5 mM leads to an "immediate" (within 10 seconds) return of the all-or-none response (see Figure 30), even after the skin has been exposed to Ca^{++}-free solution on its outer surface for an hour or longer.

Elevation of the Ca^{++} concentration in the outer solution causes a lengthening of the action potential; this is particularly true of the falling phase. Also, the refractory period is shortened in high Ca^{++} solutions. A typical set of records is shown in Figure 31. Again this is a reversible phenomenon, although it may require several minutes for full recovery from prolonged high Ca^{++} treatment. It is interesting to note that changes of Mg^{++} concentration over the same range produce no noticeable effect on the response.

e. Effect of anoxia: In all of the experiments reported above there was no attempt made to control the O_2 level in the bathing solutions. The Ringer's solution is initially saturated with 97% O_2, and it is certainly safe to say that the O_2 tension, even in prolonged experiments, does not fall below 20% (air). In the experiments to be reported in this section, however, the chamber shown in Figure 15 was used, so that it was possible to remove all of the O_2 in solution by bubbling with N_2, and then to replace O_2 by re-bubbling with air.*

Let us first consider what happens to the electrical properties of frog skin when we replace the O_2 in solution by N_2. Over a period of from one to two hours, the resting potential gradually declines, eventually reaching zero potential. If during this period the response to small steps of current is observed, it is found that for about 45 minutes there is no change in the response from that which existed in the presence of O_2. There then occurs, within a period of 5 minutes, a rise of the resistance to a new steady state

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*In the experiments reported in this section, the Ringer solution consists of 116 mM NaCl; 2.0 mM KCl; 1.8 mM $CaCl_2$; 1.0 mM NaH_2PO_4; 2.0 mM Na_2HPO_4.

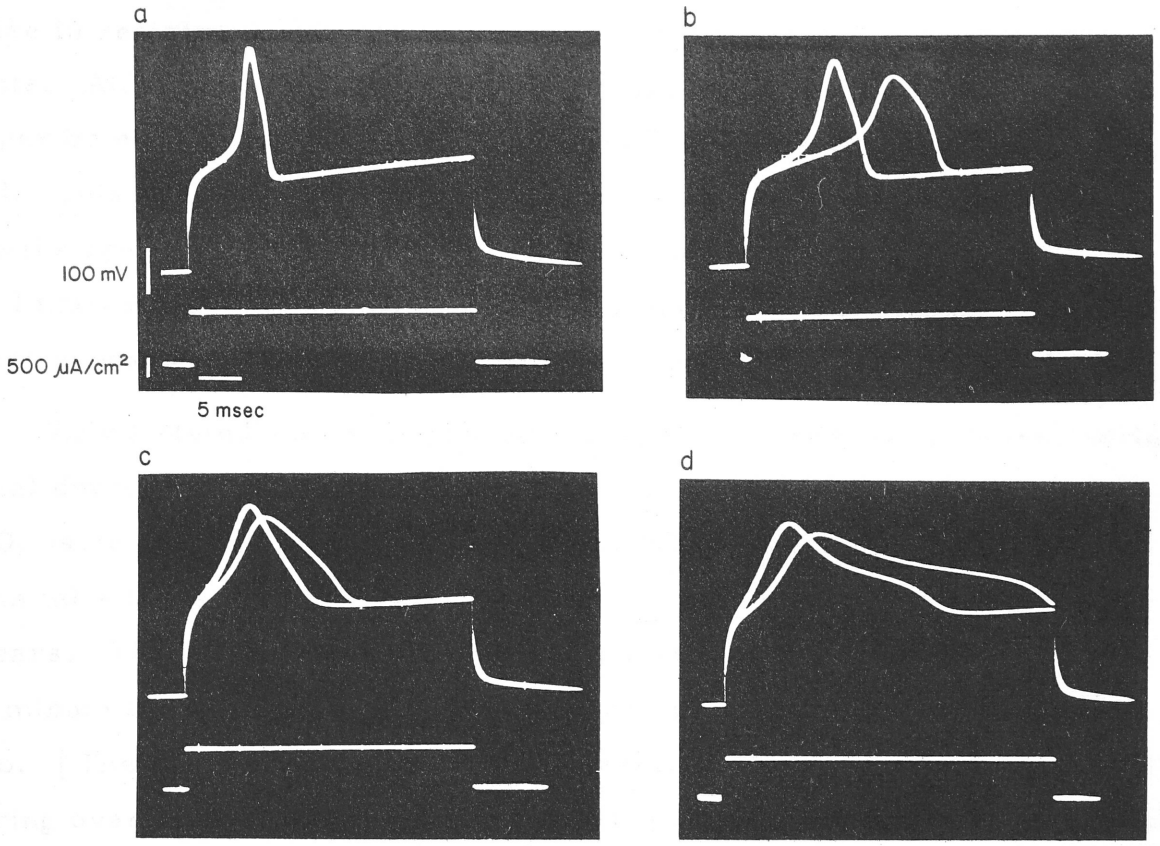

Figure 31. Effect on the action potential of isolated frog skin of increasing Ca^{++} concentration in the outside solution. In all records the inside solution is Gray-Ringer and the outside solution has 100 mM NaCl, 5 mM KCl + x mM $CaCl_2$. The Ca^{++} concentration is 2.5 mM, 10 mM, 20 mM, and 30 mM, in a, b, c, and d, respectively. In those records in which there are two tracings the interval of time between responses is 2 seconds, and the more prolonged response is the second. Note that the current necessary to produce an action potential is smaller the higher the Ca^{++} concentration. (All currents are inward.)

value of from 2 to 3 times that of the previous value. [This change occurs even before the resting potential has fallen completely to zero.] Prior to this rise in resistance, the action potential still occurs for large inward currents. After the resistance rise, an all-or-none action potential can no longer be elicited; at best a graded, overshoot response still can be evoked. This behavior is illustrated in Figure 32. In Figures 32a and 32b, we see the response of the skin in O_2 to small and large inward currents, and in Figures 32c and 32d, we see the responses after the preparation has been exposed to a nitrogen atmosphere for one hour.

As we stated above, continued exposure to nitrogen brings the resting potential down to 0, without any further changes in the skin resistance. If now, O_2 is readmitted into the system, within 3 minutes, the resistance of the skin falls to its previous value, and the full, all-or-none action potential reappears. These results are shown in Figures 32e and 32f. During this three-minute time interval, the resting potential across the skin remains at zero. [The recovery of the resting potential is a very slow process, occurring over a period of an hour or longer.] The experiment can be repeated several times. Thus, again replacing O_2 by N_2, the skin resistance rises by 2 or 3-fold and the action potential disappears. [The time required for the resistance rise in the second exposure to N_2 is much shorter than the 45 minutes necessary in the first exposure.] Readmitting O_2 causes a drop in resistance and reappearance of the action potential. We wish to emphasize that these events can take place without any concomitant changes in the resting potential, and that the oxygen effect occurs even after the skin has been in a nitrogen atmosphere for over three hours. We shall comment further on these experiments in the discussion.

We have fixated on the effect of anoxia, because of the rapid reversibility of this effect. But we wish to mention that cyanide, azide, and dinitrophenol produce the same effect. Namely, in their presence,* the

*The concentration of CN^- used was from 1 to 2 mM; the concentration of azide used was 5 mM and that of DNP was 0.5 mM.

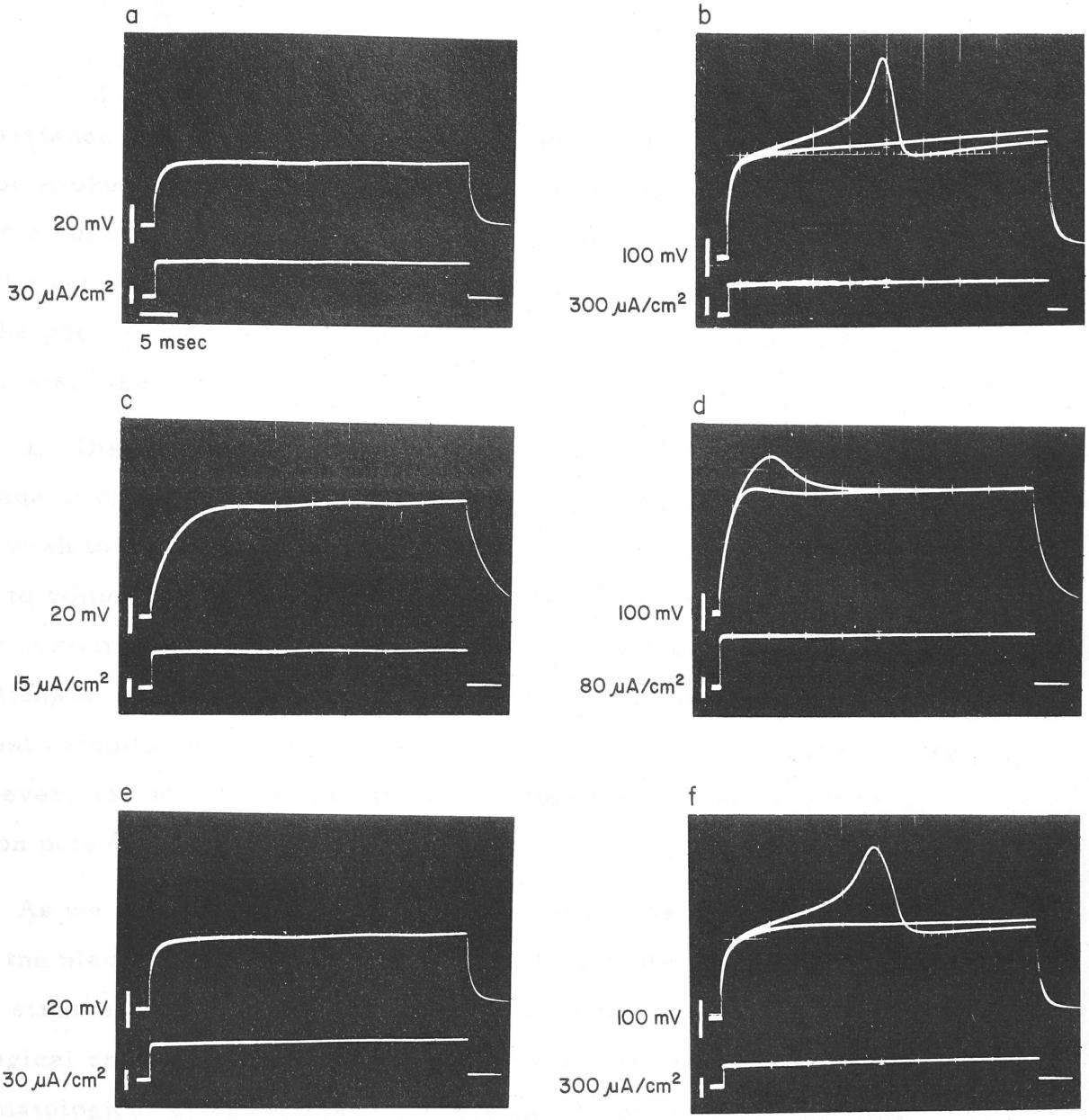

Figure 32. Effect of anoxia on the voltage response of isolated frog skin to inward current. Records a and b were taken after the skin had been exposed to oxygenated Ringer solution for over two hours. The resting potential was 20 mV. Following these records, the Ringer solution was bubbled with N_2 for 90 minutes and then records c and d were taken. The resting potential was then 0. Following these records, the Ringer solution was bubbled with O_2 for 3 minutes and then records e and f were taken. The resting potential was still 0 at this time. (In those records with two tracings, the interval between responses is 2 seconds; the first response is the larger.)

skin resistance rises by a factor of 2 or 3 and the action potential can no longer be evoked. The difficulty with these metabolic inhibitors is that it requires a considerable length of time to wash them out of the system and return the resistance to its former level and restore the action potential. Thus, the phenomenon is not as dramatic as for the simple anoxia experiments, where reversibility is obtained within 3 minutes.

3. Discussion: In the previous section we have described the phenomena of electrical excitability in isolated frog skin and toad bladder. We now wish to comment on some of our data and discuss our results with respect to somewhat related observations, as reported in the literature, on other systems; in a more general context we wish to explore the implications of our experiments with regard to our previous analysis of equivalent circuits and the ionic theory of nerve excitation. Before doing so, however, let us comment on the site within the skin and bladder of the action potential.

As we indicated earlier (page 46), the mucosal layer of epithelial cells in the bladder and the stratum germinitiva of the skin are the regions of these structures which are responsible for their electrical and physiological characteristics. This had been surmised for many years on purely histological grounds; that is, these layers of cells are the only continuous, uninterrupted regions in those organs that could possibly be acting as a significant barrier to the diffusion of ions and water. In more recent years, by the use of micropipettes, it has been established directly that the resting potential in the bladder occurs across the mucosal layer of cells (Frazier, 1962). In puncturing the bladder from the mucosal side, two steps in potential are observed; the first, corresponding to the luminal facing membrane of the mucosal cells, and the second, corresponding to the serosal facing membrane of these cells. Furthermore, the d.c. resistance of the bladder also appears across these cells, about half of the total transbladder resistance being associated with each of the two

plasma membranes.* Similar results are reported with respect to the germinitival layer in the skin (Engbaek and Hoshiko, 1957), although there has been some controversy over this point (Scheer and Mumbach, 1960).

The question arises as to which of the two plasma membranes of the epithelial cells is responsible for the action potential which we have described. Because of technical difficulties, we were unable to answer this question directly by the use of the micropipette technique. We may surmise, however, from the experiments in which the ionic composition of the solutions on the two sides of the skin were varied, that it is across the outer facing membrane in the skin (and by inference the luminal facing membrane in the bladder) that the action potential occurs, since changes in the composition of the solution bathing the inner surface produce no significant alteration in the response, while similar changes in the solution bathing the outer surface produce rapid and reversible alteration in the character of the response (see page 51 et seq.).

It is of some interest to note that the outer membrane in the skin and the luminal membrane in the bladder have been shown to be much more permeable to Na^+ than to K^+ (Koefoed-Johnsen and Ussing, 1958; Frazier et al., 1962). This is consistent with Figures 29a and 29c, where we see that the resistance of the skin rises considerably when Na^+ in the outer solution is replaced by K^+. Now if the active membrane is indeed the outer one, then, since the basis of the rising phase of the all-or-none action potential is a rise in resistance, this must mean that <u>at least</u> the Na^+

*The question might be raised as to whether the electrical and transport properties are characteristics of the cells or of the spaces between the cells. Recent electron microscopic studies have shown that the epithelial cells are very tightly fused together (Peachey and Rasmussen, 1961; Choi, 1963). Further more, studies of the osmotic movement of water across the bladder have demonstrated that the epithelial cells swell in the face of an osmotic gradient, thus indicating that the water flowing across the bladder is passing through the cells (Peachey and Rasmussen, 1961). We may therefore safely assume that the properties of interest are related to the cells themselves rather than to the intercellular spaces.

resistance must increase during the action potential, which is just the opposite of what occurs in nerve. We see further in Figure 29d that when K^+ replaces Na^+ in the outside medium, there is no longer an all-or-none response but instead a graded response which appears to be primarily a manifestation of a fall in resistance (from the high resting values in K^+ Ringer) during the current flow rather than first an increase in resistance followed by a decrease.

We have continually emphasized that the action potential in frog skin and toad bladder is a consequence of current modulation of a time variant resistance element rather than the result of a variation in any intrinsic emf. of the system. Because, as we shall argue below, this may be the general basis of electrical excitabiaity in more familiar structures (i.e. nerve and muscle), it is useful to note several other examples in the literature of responses which appear to be basically resistance phenomena. The one bearing a most striking similarity to the behavior we have described in frog skin and toad bladder is the hyperpolorizing responses in lobster muscle as reported by Reuben et al. (1961). By somewhat indirect (but rather convincing) means, these authors conclude that the action potential which they observe is a consequence of a rise and fall in transmembrane resistance during the passage of hyperpolarizing current; the figures and data which they present are very similar to some of our records [compare especially their Figure 4 with our Figure 26]. The response of the plant cell Halicystis to outward current as reported by Blinks (1936 b) also bears a close resemblance to the basic response we have described. While Blinks does not address himself to the problem as to whether he is in fact dealing with a time variant resistance phenomenon, this would appear to be a reasonable conclusion from his data.

In the frog node of Ranvier bathed by isotonic KCl there occurs a response reported by Mueller (1959) which also appears to be the result of resistance variation. In this case, however, the resistance first falls and

then rises rather than the reverse, which occurs in our systems. The phenomenon is as follows: hyperpolarizing current is passed across the node until its potential is brought back to approximately the resting value (- 60 mV.). If now a short cathodal (depolarizing) current is passed across the node, then superimposed on the hyperpolarizing current, the potential rises to a value always less than 0, and then falls back to the resting value. Although Mueller does not interpret this action potential as being due to resistance changes, his data are clearly consistent with such an interpretation.

Finally, we may mention some recent interesting experiments by Mueller et al. (1962 a, b) on in vitro bimolecular lipid films. If certain (as yet unidentified) substances are introduced into these films, the following behavior is described: When sufficient current is passed across this membrane, a threshold value of potential is reached from which the potential rises from its previous level to a new steady state value, and remains at this value so long as current flows. These authors have shown that the basis of this potential rise is an increase in the membrane resistance. Note, however, that the potential does not fall back to its sub-threshold level so long as current is flowing. Thus, the response of these films corresponds phenomenologically to the first half (rising phase) of the action potential in frog skin and toad bladder. [Compare also the behavior of Valonia (Blinks, 1936 a).]

Let us now consider some of the implications of the anoxia experiments which we have previously described (page 53 et seq.). The first point which deserves emphasis is the independence of the resting potential across the skin and the resistance response. We have seen that prolonged anoxia can bring the resting potential down to zero and approximately double the resting resistance; in this state the skin is not excitable. Upon the readmission of oxygen to the system, the resistance quickly decreases, with a simultaneous return of excitability, without any change in the resting potential. Without becoming involved in the various hypotheses put forth to explain the resting

potential across the skin (Linderholm, 1952; Koefoed-Johnson and Ussing, 1958), we can assert from our data that quite dramatic changes in the resistance of this system can occur without any noticeable alteration in its intrinsic emf. properties.

The second point of interest is that the resistance of the skin (the plasma membranes) is a function of metabolic activity. The rapidity with which the resistance changes when oxygen is readmitted to the previously anoxic system precludes any significant changes of the ionic composition within the cell and clearly demonstrates that the physical state of the cell membrane can be altered by metabolic events. Furthermore, and this brings us to the third point, it would appear that the changes in the membrane produced by metabolism are related to the changes that occur during electrical stimulation. Thus, in the normal oxygenated skin there occurs, at a threshold potential, a regenerative rise and fall of the resistance. If, however, the system has been previously brought into the high resistant state by anoxia, then there is no further regenerative increase to a higher state during current flow. We might glibly infer from this that the same site in the plasma membrane is responsive to both metabolic and electrical stimulation.*

To conclude our discussion, we turn to a consideration of the implications that our studies on the action potential in frog skin and toad bladder have with regard to the equivalent circuit representation of the electrical events occurring in nerve. We recall that the electrical properties of the nerve plasma membrane can be described by a two-

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*Blinks (1949, 1955) has observed that the resistance across the plant cell Halicystis rises when O_2 is removed from the surrounding solution. As we mentioned earlier, Halicystis also shows electrical excitability bearing a close resemblance to the response we have been describing. Blinks also suggests that O_2 and electrical stimulation may be acting through a common mechanism.

branched equivalent circuit of the type shown in Figure 10, one branch consisting of a sodium emf. and sodium conductance in series, and the other branch consisting of a potassium emf. and potassium conductance in series (Figure 33a). The two emf.'s are invariant (being determined by the fixed boundary conditions) but the conductances are complicated functions of the membrane potential, Ψ, and time. We further recall (see page 34 et seq.) that we were able to show that such a circuit can apply both to a homogeneous and heterogeneous membrane, despite the fact that in a homogeneous membrane any potential variation, in the absence of current flow, must be due to a time-variant emf. rather than to time-variant conductances. It was this ambiguity in the meaning of the equivalent circuit as applied to nerve that motivated us to consider the frog skin and toad bladder, where we were able to demonstrate unambiguously that the action potentials are the result of a time-variant conductance. If we choose to draw an equivalent circuit for these systems, it will consist of a single branch with an emf. and conductance in series, where the emf. is invariant and the conductance is time-variant (Figure 33b).

Let us assume now that the axonal membrane is indeed a mosaic membrane consisting of separate regions of exclusive permeability to either sodium or potassium. This will mean, in view of our previous discussion (page 40), that there are always local currents flowing within the membrane even in the resting state; that is, in the absence of any net current flow across the membrane. In other words, the circular current flow depicted in Figure 31a has real physical meaning. Now compare this with the circuit in Figure 31b for the frog skin and toad bladder. Here, there is no current flow in the membrane unless it is introduced by means of external electrodes; this, however, can always be done, and is in fact the manner in which our experiments were conducted. Suppose that we pass a just sub-threshold current across the preparation and define this state of the system as the resting state. Then, from a phenomenological point-of-view, the resting state in the nerve (as depicted in Figure 33a) is basically the same as the resting state in the skin and bladder (Figure 33b); in both cases, there are

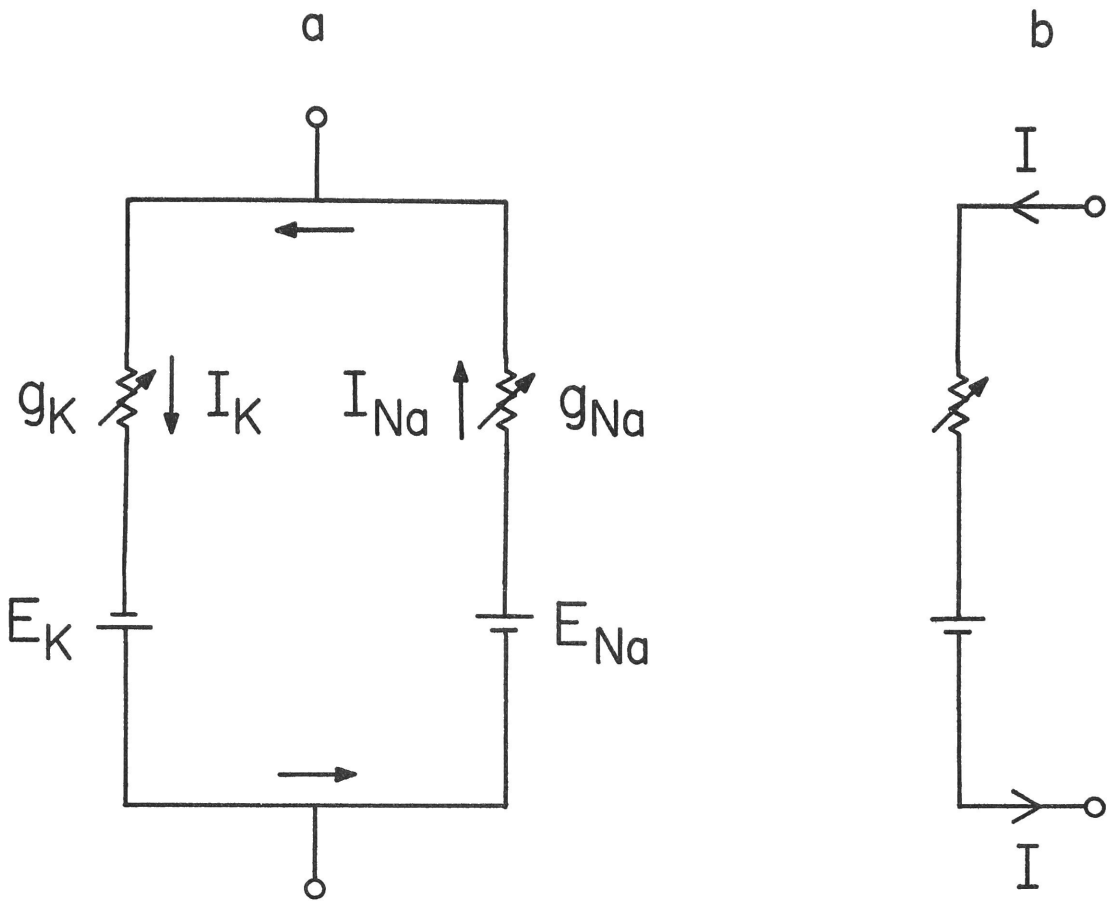

Figure 33. (a) Equivalent circuit for the axonal membrane.
(b) Equivalent circuit for frog skin and toad bladder.

time-variant resistances across which a resting current is flowing.

With the above remarks in mind, let us turn our attention back to Figure 21d. In this figure, we see that a small step of current of short duration superimposed on a constant sub-threshold current is able to trigger a full-blown action potential, and that this action potential occurs after the small step of current has been removed; remember, however, that the sub-threshold current is still flowing during this time, and is absolutely essential for a response to occur. On the other hand, if we were unaware of this sub-threshold current, we would regard the record in Figure 21d as follows: "If frog skin is stimulated by a step of current of sufficient magnitude and proper polarity then an action potential occurs across this structure in the absence of any current flow." Substitution of the word "nerve" for "frog skin" in the above sentence gives a description of the electrical excitability of nerve. The point we are trying to make is this: If the axonal membrane is a mosaic structure as we described it above, then the action potential, which occurs in the absence of any external current flowing across the membrane, is not due to a variation in any emf. of the system but is the result of a resistance modulation of the local currents flowing within the membrane. The local currents in the nerve would then be analogous to and performing the same role as the sub-threshold current in the action potential of frog skin as seen in Figure 21d.

The arguments presented above are not intended as a "proof" that the plasma membrane of the nerve is a mosaic structure of selective permeable regions and that the action potential is the result of time-variant resistances. What we have tried to do, however, is to make reasonable and plausible this interpretation by drawing a parallelism between the nerve and the frog skin-toad bladder systems, where the action potential is unquestionably a resistance phenomenon. We may mention that the idea of the plasma membrane being a non-homogeneous structure is by no means a new one. From the permeability properties of the plasma membrane, it has generally been concluded that there are certain regions which are permeable

to lipid soluble molecules and other regions permeable to water soluble molecules (Hober): that these two broad classes of regions should be further specialized is certainly not unreasonable. We may finally mention that if one accepts the Hodgkin and Huxley analysis of the action potential in the squid axon (1952 d), then the fact that the sodium and potassium conductances can change quite independently of each other is highly suggestive that the sodium and potassium ions do not pass through the same regions in the membrane.

APPENDIX I

We shall prove that for a homogeneous fixed-charge membrane separating two solutions of equal total concentration of univalent electrolytes, the potential within the membrane in the steady state will be a linear function of x ("constant field").

Since the concentrations on the two sides are equal, the Donnan potentials cancel each other, and we are only concerned with the concentrations and potential following the Donnan "jumps". Our notation will be essentially that used by Teorell (1951). [The same notation is followed in the text.] Writing the flux equations (1a) and (1b) in slightly different form, we have:

$$\phi_j^+ = - u_j\left(RT \frac{dc_j^+}{dx} + Fc_j^+ \frac{d\psi}{dx}\right) = - u_j A_j$$

$$\phi_k^- = - v_k\left(RT \frac{dc_k^-}{dx} - Fc_k^- \frac{d\psi}{dx}\right) = - v_k B_k$$

and letting $A = \sum_j A_j$ and $B = \sum_k B_k$ we obtain:

$$A = RT \frac{dc^+}{dx} + Fc^+ \frac{d\psi}{dx} \qquad \text{(I. 1a)}$$

$$B = RT \frac{dc^-}{dx} - Fc^- \frac{d\psi}{dx} \qquad \text{(I 1b)}$$

where $c^+ = \sum_j c_j^+$ and $c^- = \sum_k c_k^-$ at any point x.

Introducing the condition of electric neutrality:

$$c^+ + \omega\bar{X} = c^-$$

where $\omega\bar{X}$ is the concentration of fixed-charge, and adding (1a) and (1b), we obtain:

$$(A + B) = RT \frac{d}{dx}(2c^+ + \omega\bar{X}) - F\omega\bar{X} \frac{d\psi}{dx} . \qquad \text{(I. 2)}$$

Integrating from $x = 0$ $(\psi = 0)$ to $x = \delta$ $(\psi = \Psi)$ and remembering that $c_0^+ = c_\delta^+$, we get:

$$(A + B)\delta = - F\omega\bar{X}\Psi \tag{I. 3}$$

Integrating (I. 2) from $x = 0$ $(\psi = 0)$ to $x = x$ $(\psi = \psi)$, we find:

$$c^+ = \frac{F\omega\bar{X}}{2RT}\left(\psi - \frac{x}{\delta}\,\Psi\right) + c_0^+ \tag{I. 4}$$

Subtracting (I. 1b) from (I. 1a) we have:

$$(A - B) = RT\,\frac{d(c^+ - c^-)}{dx} + F(c^+ + c^-)\frac{d\psi}{dx} = F(2c^+ + \omega\bar{X})\,\frac{d\psi}{dx} \tag{I. 5}$$

And substituting (I. 4) into (I. 5) and rearranging we have:

$$(A - B)\,\frac{dx}{d\psi} = \frac{F^2\omega\bar{X}}{RT}\,\psi - \frac{F^2\omega\bar{X}\Psi}{RT\delta}\,x + F(2c_0^+ + \omega\bar{X}) \tag{I. 6}$$

We wish to solve (I. 6) for $(A - B)$. For notational convenience let:

$$\alpha = \frac{F^2\omega\bar{X}}{RT}$$

$$\beta = - \frac{F^2\omega\bar{X}\Psi}{RT\delta}$$

$$\gamma = F(2c_0^+ + \omega\bar{X})$$

Changing variables by writing:

$$v = \alpha\psi + \beta x + \gamma \tag{I. 7}$$

differentiation leads to:

$$\frac{dv}{d\psi} = \alpha + \beta\,\frac{dx}{d\psi} \tag{I. 8}$$

and comparing (I. 7) and (I. 8) with (I. 6) we can write:

$$\frac{dx}{d\psi} = \frac{v}{A - B} = \frac{1}{\beta}\left(\frac{dv}{d\psi} - \alpha\right)$$

and rearranging we have finally:

$$\frac{dv}{\alpha + \frac{\beta}{A - B} v} = d\psi \tag{I. 9}$$

The integration of (I. 9) will be between $v = \gamma$ and $v = \alpha\Psi + \beta\delta + \gamma$. In order to perform this integration, we must exclude for the time being the particular value:

$$(A - B) = - \frac{\beta\gamma}{\alpha} \tag{I. 10}$$

since for this value of $(A - B)$, $\alpha + \frac{\beta}{A - B} v$ becomes infinite at $v = \gamma$. Remembering this restriction, integration of (I. 9) gives:

$$\ln \frac{\alpha(A - B) + \alpha\beta\Psi + \beta^2\delta + \beta\gamma}{\alpha(A - B) + \beta\gamma} = \frac{\beta}{A - B} \Psi$$

and since $\alpha\beta\Psi + \beta^2\delta = 0$, we obtain:

$$\beta\Psi = 0 \tag{I. 11}$$

Since, however, β and Ψ can assume any finite value we choose, relation (I. 11) is absurd. But equation (I. 11) results from any value of $(A - B)$, except that which is given in (I. 10). This must mean that the value of $(A - B)$ which we excluded, (I. 10), was the correct value, i. e.:

$$(A - B) = - \frac{\beta\gamma}{\alpha} = \frac{F\Psi}{\delta} (2c_0^+ + \omega\bar{X})$$

Introducing this into (I. 6) and rearranging we get:

$$\left[\frac{F\omega\bar{X}}{RT} (\psi - \frac{x\Psi}{\delta}) + 2c_0^+ + \omega\bar{X} \right] \frac{d\psi}{dx} = \frac{\Psi}{\delta} (2c_0^+ + \omega\bar{X}) \tag{I. 12}$$

By inspection, the solution of (I. 12) is:

$$\psi = \frac{x}{\delta} \Psi \tag{I. 13}$$

i. e., the potential is a linear function of x. We may note that the only restriction in our derivation was that a steady state exists. Thus, the result is valid with finite current flowing across the membrane.

APPENDIX II

We wish to determine the steady state values of g_j, $R_{int.}$, and Ψ_D for the case of a homogeneous uncharged membrane separating two solutions of equal total concentration of univalent electrolytes. In order to do this, we must calculate the ionic profiles within the membrane; i.e., we must obtain an expression for the concentration of each ion as a function of x. Once this is accomplished, the evaluation of the desired quantities is merely a matter of integration.

We start with the flux equation for an arbitrary cation:

$$\phi_j^+ = - u_j \left(RT \frac{dc_j^+}{dx} + Fc_j^+ \frac{d\psi}{dx} \right) \qquad \text{(II. 1)}$$

Since the concentrations on the two sides of the membrane are equal, $\frac{d\psi}{dx}$ is a constant (Appendix I). If we take the membrane thickness as δ and let $\psi = 0$ at $x = 0$ and $\psi = - \Psi$ at $x = \delta$, then $\frac{d\psi}{dx} = - \frac{\Psi}{\delta}$ and (II. 1) becomes:

$$\phi_j^+ = - RTu_j \frac{dc_j^+}{dx} + Fu_j c_j^+ \frac{\Psi}{\delta} \qquad \text{(II. 2)}$$

Integrating (II. 2) from $x = 0$ $[c_j^+ = (c_j^+)_0]$ to $x = \delta$ $[c_j^+ = (c_j^+)_i]$ we get:

$$\phi_j^+ = \frac{F\Psi}{\delta} u_j \frac{(c_j^+)_0 - (c_j^+)_i \, e^{- \frac{F\Psi}{RT}}}{1 - e^{- \frac{F\Psi}{RT}}} \qquad \text{(II. 3)}$$

Integrating (II. 2) from $x = 0$ to $x = x$ we obtain:

$$c_j^+ = \frac{F\Psi u_j (c_j^+)_0 - \delta \phi_j^+ \left(1 - e^{- \frac{F\Psi}{RT} \frac{x}{\delta}} \right)}{F\Psi u_j \, e^{- \frac{F\Psi}{RT} \frac{x}{\delta}}} \qquad \text{(II. 4)}$$

Substituting (II. 3) into (II. 4) gives:

$$c_j^+ = \frac{(c_j^+)_0 - \dfrac{(c_j^+)_0 - (c_j^+)_i\, e^{-\frac{F\Psi}{RT}}}{1 - e^{-\frac{F\Psi}{RT}}}\left(1 - e^{-\frac{F\Psi}{RT}\frac{x}{\delta}}\right)}{e^{-\frac{F\Psi}{RT}\frac{x}{\delta}}} \qquad \text{(II. 5)}$$

Letting,

$$\xi_x \equiv e^{\frac{F\psi}{RT}} \equiv e^{-\frac{F\Psi}{RT}\frac{x}{\delta}}$$

$$\xi \equiv e^{-\frac{F\Psi}{RT}}$$

equation (II. 5) can be written in a more condensed form:

$$c_j^+ = \frac{[(c_j^+)_i \xi - (c_j^+)_0](\xi_x - 1)}{\xi_x(\xi - 1)} + \frac{(c_j^+)_0}{\xi_x} \qquad \text{(II. 6a)}$$

An analogous treatment starting with the flux equation for an anion gives:

$$c_k^- = \frac{[(c_k^-)_i - \xi(c_k^-)_0](1 - \xi_x)}{(1 - \xi)} + \xi_x (c_k^-) \qquad \text{(II. 6b)}$$

With (II. 6a) and (II. 6b) we can now directly calculate all of the relevant quantities of the two types of equivalent circuits:

$$g_j^+ \equiv \frac{1}{\int_0^\delta \frac{dx}{Fu_j c_j^+}} = \frac{u_j F^2 \Psi}{RT\delta} \frac{(c_j^+)_i \xi - (c_j^+)_0}{(1 - \xi)\ln \frac{(c_j^+)_i \xi}{(c_j^+)_0}} \qquad \text{(II. 7a)}$$

$$g_k^- \equiv \frac{1}{\int_0^\delta \frac{dx}{F v_k c_k^-}} = \frac{v_k F^2 \Psi}{RT\delta} \frac{(c_k^-)_0 \xi - (c_k^-)_i}{(1-\xi) \ln \frac{(c_k^-)_0 \xi}{(c_k^-)_i}} \qquad \text{(II. 7b)}$$

$$R_{int.} \equiv \int_0^\delta \frac{dx}{F(U+V)} = \frac{RT\delta}{F^2\Psi} \frac{(1-\xi)}{\sqrt{b^2-4ac}} \ln \frac{\xi(U_i+V_0) + (U_0+V_i) - \sqrt{b^2-4ac}}{\xi(U_i+V_0) + (U_0+V_i) + \sqrt{b^2-4ac}} \qquad \text{(II. 8)}$$

$$\Psi_D \equiv \frac{RT}{F} \int_0^i \frac{d(U-V)}{(U+V)} = \Psi + \frac{RT}{F} \frac{b}{\sqrt{b^2-4ac}} \ln \frac{\xi(U_i+V_0) + (U_0+V_i) - \sqrt{b^2-4ac}}{\xi(U_i+V_0) + (U_0+V_i) + \sqrt{b^2-4ac}} \qquad \text{(II. 9)}$$

where,

$$U_0 = \sum_j u_j (c_j^+)_0 ; \quad U_i = \sum_j u_j (c_j^+)_i ; \quad V_0 = \sum_k v_k (c_k^-)_0 ; \quad V_i = \sum_k v_k (c_k^-)_i$$

$$a = (V_0 - V_i); \quad b = (U_0 + V_i) - \xi(U_i + V_0); \quad c = \xi(U_i - U_0)$$

We note from these expressions that, in general, g_j, $R_{int.}$, and Ψ_D are voltage dependent. It is interesting, however, that for the special case where there is only one anion (or cation) in the system, then, since $V_i = V_0 = V$, equation (II. 9) becomes:

$$\Psi_D = \frac{RT}{F} \ln \frac{U_i + V}{U_0 + V}$$

for all Ψ. That is, Ψ_D is independent of voltage, which we asserted in the footnote on page 41.

APPENDIX III

The two-layered sandwich membrane which is discussed in the text is in many ways quite similar to the solid state p-n junction. In fact, if the solution on the two sides of the membrane is distilled water instead of a KCl solution, then, as has been pointed out by Mauro (1962), there is a distinct analogy between the two systems. Because of this, it is of some interest to compare our treatment of the sandwich membrane with certain aspects of the analysis of the p-n junction as carried out by Shockley (1949).

The first thing that we wish to explain is the difference in the starting point of our treatment as compared to Shockley's. In deriving the Ψ - I characteristics of the p-n junction, Shockley starts with the assumption that virtually all of the potential difference between the terminals of the device appears across the transition region; in other words, the IR drops in the bulk of the material make a negligible contribution to the potential difference. In our analysis, we have tried to indicate the basis for this assumption. As we have shown [see equations (37) and (38)], when the fixed-charge density is high, the IR contributions do become negligible, and the potential difference across the system appears primarily across the transition region. We have in fact seen that the basis for this potential difference is the shifting of the Donnan emf.'s in the junction region.

The second point that we wish to discuss is the differences that arise between a weak electrolyte system (H^+ and OH^- in the sandwich membrane bathed by pure H_2O or holes and electrons in the solid state device) and the strong electrolyte system (KCl) with which we have dealt. In the case of the weak electrolyte, the boundary conditions are maintained by the recombination of the H^+ and OH^- ions, so that the voltage response of the device is independent of the membrane thickness. [In the Shockley paper the p and n regions are taken as infinite in extent.] For the

strong electrolyte case, on the other hand, the boundary conditions are established by the Donnan equilibria at the two solution-membrane interfaces, and consequently the membrane thickness is an important parameter in determining the magnitude of π resulting from a given I. The physical basis for this is that for a given I, the thicker the membrane the larger the concentration difference that can be maintained between i1m and 1m (and between i2m and 2m), and hence the larger the Donnan emf. that can develop at the junction.

A further difference between the strong and weak electrolyte situations is that in the former case the steady state Ψ - I characteristic is independent of the distance between the positive and negative fixed-charge lattice (so long as we neglect any IR drop in this region), while in the latter case, the degree of coupling of the two lattices is crucial. Only when the lattices are tightly coupled can the concentration, c_i, of H^+ and OH^- at the center of the junction be perturbed and thus give rise to a change in the Donnan emf. When the lattices are apart, then, because of recombination, c_i remains fixed; such a junction will behave as a pure ohmic device. Since there is no recombination, by definition, in a strong electrolyte, the steady state π resulting from a given I will clearly not be affected by the size of the middle compartment between the two lattices. Of course, the time required to reach a steady state will be affected by this parameter.

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